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Public Health and Marine-Hospital Service of the United States

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STUDIES UPON ANAPHYLAXIS

WITH SPECIAL REFERENCE TO THE ANTIBODIES CONCERNED

BY

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AND

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STUDIES UPON ANAPHYLAXIS WITH SPECIAL REFERENCE TO THE ANTIBODIES CONCERNED.[a]

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INTRODUCTION.

During the past year the interest in the phenomenon of anaphylaxis has been unusually active, and much work has been done which is of value in increasing our knowledge of this subject. While the value of some of this work remains doubtful, because of insufficient time for confirmatory work and some because of conflicting results of various workers, still, the well-established facts brought out remain sufficient to add materially to our knowledge of the mechanism of the anaphylactic reaction. Without attempting to give a review of the work of the year, it may be well to mention a few of the well-defined and striking facts brought out.

To Doerr and Russ (11) is due the credit of having first demonstrated the application of definite quantitative methods to the study of anaphylaxis and of calling attention to passive anaphylaxis as the most promising field for such quantitative study.

It had already been demonstrated that the serum of a sensitive rabbit injected into a fresh guinea pig, caused the latter, after 24 hours or more, to become sensitive to the injection of the specific antigen proteid.

Doerr and Russ (12) undertook a *quantitative* study of this passive sensitizing property of sensitive serum, using three methods:

(1) A series of guinea pigs were injected intraperitoneally with the *same amount of serum* from a rabbit sensitized by repeated injections of foreign serum, and tested after 24 hours by intravenous injections of *varying amounts of antigen* serum, to determine the minimum lethal dose of the latter.

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[a] Manuscript submitted for publication, May 10, 1910.

(2) A series of guinea pigs were injected intraperitoneally with *varying amounts of sensitive rabbit serum* and tested after 24 hours by the intravenous injection of a *fixed dose of antigen* serum, to determine the minimum amount of sensitive serum capable of fully sensitizing a guinea pig within this time.

(3) A series of guinea pigs were injected *simultaneously with a fixed quantity of sensitive rabbit serum, plus varying amounts of its specific antigen,* and then tested in 24 hours by intravenous injection of a massive dose of antigen serum, thus determining the least amount of antigen sufficient to neutralize the sensitive serum within the body of the guinea pig.

Their demonstration, by these three methods, of a definite quantitative relation between antigen and sensitive antibody must be considered a distinct landmark in the progress of the study of anaphylaxis, a point of departure for much of the subsequent work.

The application of these methods of quantitative estimation of hypersusceptibility has made possible the comparative determination of the amount of antibody in the serum of animals of the same species treated in different ways (by single or repeated injections) as well as the comparative amount of antibody developed by the same treatment in animals of different species. It has made possible, by more exact estimation of the quantitative relations between sensitizing and intoxicating dose, a more complete demonstration than heretofore presented of the fallacies in the reported separation of the intoxicating from the sensitizing portion of proteids by such means as heat and fractional precipitation, confirming those who have held that the same serum constituent effects both sensitization and intoxication, a view presented by Rosenau and Anderson (38) in their first publication on the subject of anaphylaxis. It has made possible the study of the quantitative relation of precipitin to sensitizing antibody in specific antiserum, a relation which is still the subject of much dispute.

It has also been demonstrated by such methods that an animal of a species comparatively insusceptible to anaphylactic shock, may yet carry in its blood even *more* of the sensitive antibody than an animal of another species which is highly susceptible to anaphylactic shock. For instance, a rabbit sensitized by one or more injections of horse serum may react very slightly, seldom fatally, to another injection of horse serum; yet the serum of this rabbit, injected into fresh guinea pigs, renders these animals *more sensitive* than would the same amount of serum from a sensitive guinea pig, although the latter would itself react very violently (usually fatally) to a reinjection of horse serum.

Further work has been done by Doerr and Russ (12), Braun (10), and others, to determine the time (fixation-period) that is necessary to elapse after the injection of sensitive serum into a fresh animal before the latter becomes sensitive. It has been shown that the time necessary for the development of a high degree of sensitiveness is about four hours after intravenous injection, and more than 12 hours after intraperitoneal injection.

On the other hand it has been reported by several observers (Richet (37), Doerr and Russ (12), Friedberger (16, 17), Biedl and Kraus (9)) that they have at last succeeded in producing *immediate* anaphylactic reaction in *fresh* guinea pigs by injecting suitable mixtures of antigen and anaphylactic antibody.

It has been demonstrated by Auer and Lewis (4, 5) that the immediate cause of death in sensitive guinea pigs reacting to a second injection of antigen is asphyxia, due to tetanic contraction of the smooth muscles of the bronchioles, and that this contraction is probably peripheral rather than central in origin. These findings have been confirmed by Anderson and Schultz (2) working on guinea pigs, and by Friedberger (16) with rabbits. Marked fall of blood pressure during anaphylactic shock has been demonstrated by Auer and Lewis and by Anderson and Schultz in guinea pigs, and by Biedl and Kraus (8) and Arthus (3) in dogs. Pfeiffer (35, 36) has insisted that a marked fall of temperature is a constant symptom of anaphylactic shock; while this has been confirmed as regards severe anaphylactic shock, we do not consider it satisfactorily proven that fall of temperature is, as Pfeiffer claims, the most constant and delicate symptom of anaphylactic reaction.

Biedl and Kraus (8) and Arthus (3) have shown decreased coagulability of the blood of dogs during anaphylactic shock, and Friedberger (17) has shown the same in guinea pigs. Biedl and Kraus (8) have also called attention to the identity of the symptoms of peptone poisoning and anaphylactic shock in dogs, and have reported that the reaction of a sensitive dog to intravenous injection of peptone renders the animal antianaphylactic.

The claim that the complement content of the blood is constantly and markedly decreased in the course of anaphylactic reaction has not been proven beyond dispute, although the results of various observers (Friedemann (22), Friedberger and Hartoch (20), Tsuru (47), Scott (45), Sleeswijk (46), Doerr and Moldovan (13), Michaeli and Fleischmann (27), have strongly suggested that the complement of the blood plays an essential part in the reaction.

Anaphylactic shock on the injection of a massive dose of antigen may be prevented in sensitized guinea pigs, as shown by Besredka (7) and by Lewis (25) and others by the injection of a *small* amount of the specific antigen four hours or more previously, in such a way that it may be *slowly* absorbed, preferably subcutaneously. This is taken to be exactly analogous to the fact shown by Doerr and Russ (12) and confirmed by our own work, that a very small amount of antigen injected along with sensitive serum into a fresh guinea pig will prevent the latter from becoming sensitive within 24 hours. These may be called the *specific* methods of preventing anaphylactic shock, explained by the saturation of the sensitive antibody by specific antigen, the reaction, however, occurring too slowly to give rise to explosive manifestations.

Nonspecific agents for preventing anaphylactic shock have not proven so constant nor so complete in their action. Auer and Lewis (4) have shown that the preliminary administration of atropine greatly modifies anaphylactic shock in the guinea pig. Anderson and Schultz (2) have confirmed this and have reported that they obtained better results by maintaining artificial respiration with pure oxygen, along with chloral hydrate and adrenalin.

Many attempts have been made to demonstrate *in vitro* a specific reaction between the serum or cellular tissues of sensitive animals and the sensitizing antigen, but while some observers have previously claimed to have demonstrated such reactions to their own satisfaction, their results have never been universally convincing. Recently, however, Schultz (44), working in the Hygienic Laboratory, has shown that horse serum acting *in vitro* upon excised smooth muscle fiber from a guinea pig sensitized to horse serum, causes a contraction very much more marked than when acting, under similar conditions, upon the same tissue from a normal guinea pig. This reaction must be considered of the utmost importance in a conception of anaphylaxis, showing as it does,

(1) That the reaction taking place *in vitro* between horse serum and specifically sensitive smooth muscle fiber is an *acceleration and exaggeration* of the reaction between horse serum and normal smooth muscle fiber;

(2) That the contraction of the smooth muscle fiber of a sensitive guinea pig may be caused by the *direct (peripheral) stimulation* of horse serum, confirming the opinion of Auer and Lewis and of Anderson and Schultz, that the muscular contractions previously noted as characteristic of anaphylactic shock may be peripheral in origin.

Friedberger (15) has recently called attention to some reactions *in vitro* demonstrated several years ago by himself, Moreschi, and Bezzola (18, 21), and has correlated these reactions with the anaphylactic reaction in the body. Their demonstration was briefly as follows: A hemolytic series of sheep's corpuscles + goat's hemolytic amboceptor + complement, is standarized to determine the minimum amount of hemolytic amboceptor and of complement necessary for complete hemolysis. On introducing a small amount of rabbit serum which contains specific precipitins (anaphylactic antibody) for *goat* serum, it is found that the same amount of amboceptor causes more rapid hemolysis and that a smaller amount of amboceptor is needed for complete hemolysis. It is necessary to remove the excess of precipitating serum after it has become bound to the sensitized blood corpuscles, as otherwise hemolysis is *restrained.* While the complicated nature of this reaction makes its interpretation somewhat obscure, Friedberger's conclusion seems justified, namely, that the effect of the precipitating (sensitive) rabbit serum is to *activate* its antigen (which in this case is the hemolytic goat serum), enabling it to carry out more quickly and completely its specific combination with blood corpuscles on one side and complement on the other. The restraining action of an excess of the precipitating serum is thought to be due to deflection of the complement. More recent researches by Moreschi (28, 29), Friedberger and Hartoch (19) indicate that a similar acceleration of the reaction of hemagglutination, bacterial agglutination, bacteriolysis, and phagocytosis (opsonins), takes place under similar conditions.

Friedberger (17) has recently shown that the serum of rabbits treated several times with relatively large doses of sheep serum is capable by itself, when injected intravenously, of producing anaphylactic shock in guinea pigs sensitized to sheep serum; also, less constantly, causes immediate anaphylactic shock in *normal* guinea pigs. From this he argues that the antisheep rabbit serum contains a "rest" of uneliminated sheep serum, giving it, when injected into a guinea pig, the action of an antigen-antibody mixture.

The experiments, as outlined, are open to several criticisms.

(1) Normal rabbit serum, as Friedberger states, will, in dose of 4 to 5 c. c. intravenously, kill a normal guinea pig in 10 minutes with symptoms of anaphylactic shock. Although he states that one-twentieth of this dose of precipitating rabbit serum will cause anaphylactic shock in normal guinea pigs, the least dose shown by his protocols to have produced this effect is 2 c. c.

(2) Guinea pigs sensitized to sheep serum may be expected to give a slight reaction to rabbit serum when injected intravenously in such large amounts as 2 c. c. or more. Though "controls" are given against this, they are not thoroughly satisfactory, as in all cases the mixtures of normal rabbit serum into such sensitive guinea pigs was followed, within 30 minutes, by injection of sheep serum.

(3) Rabbit serum, which strongly precipitates sheep serum, is apt to have a slight precipitating effect also upon guinea pig serum. The effect of this rabbit immune serum on sensitive or normal guinea pigs may therefore possibly be attributed to its action on guinea pig proteid, since the presence of precipitins for guinea pig proteid probably connotes the presence of anaphylactic antibody capable of acting upon this proteid.

(4) Guinea pigs receiving a dose of this rabbit precipitating serum are quite constantly *sensitive,* 24 hours later, to sheep serum, whereas

if sheep serum was present in the rabbit immune serum we would expect these guinea pigs to be antianaphylactic to sheep serum. As a matter of fact, though, guinea pigs which had reacted to this rabbit immune serum one day were found less susceptible to the same rabbit immune serum the next day.

In short, the objections to these results are that they were obtained by intravenous injection of considerable amounts of *heterologous* (rabbit) serum, which is itself somewhat toxic to the guinea pig; and that a group reaction (between antisheep antibody and guinea pig proteid) is not sufficiently excluded. Still it is not at all impossible that a small amount of sheep serum remaining uneliminated in the rabbit's circulation is the true explanation of these observations, especially in view of the following observations:

In the same paper Friedberger reports a single instance in which he caused immediate and fatal anaphylactic shock in a 220-gram normal guinea pig by the intravenous injection of 5 c. c. of serum from a guinea pig which had received two injections of sheep serum (last injection 0.5 c. c. 36 days previously); considering the very *large* amount of serum injected and several negative results obtained with smaller amounts (3 c. c.) of this and other similar serum, this single experiment needs confirmation before it can be accepted as demonstrating the point.

Friedberger further reports that he has caused anaphylactic shock in normal guinea pigs by the intravenous injection of 4 to 5 c. c. of blood taken from guinea pigs during anaphylactic shock. The guinea pigs from which the blood was taken had usually received *several* injections of sheep serum as *sensitizing* doses, and received the *intoxicating* dose of sheep serum *intravenously*. The conditions were therefore most favorable for the immediate admixture *in corpore* of a large amount of antigen with abundant anaphylactic antibody. His experiments, therefore, differ *quantitatively* from most of the previous experiments with the blood of intoxicated anaphylactic animals—a difference which explains the negative results of previous experiments of the same kind.

EXPERIMENTAL WORK.

The experimental work which follows is chiefly a study of the anaphylactic antibody (allergin) designed as a study of the principles of anaphylaxis rather than of its details. We have given especial attention to the quantitative relations existing between allergin and its specific antigen, also to the relation of allergin to the conditions of hypersusceptibility, antianaphylaxis, and immunity.

We have confined our studies chiefly to the manifestations of anaphylaxis in the guinea pig because, while the manifestations in the guinea pig are admittedly very different from those in other animals, the guinea pig is, we believe, on account of its very constant and extreme susceptibility, the most suitable experimental animal for a study of principles which can later be applied to the anaphylactic manifestations in other animals. We have also confined ourselves to horse serum and eggwhite as antigens, because the lack of toxicity of these proteids for *normal* animals excludes the experimental errors due to the use of more toxic substances.

Rosenau and Anderson (41), in previous publications, have used the term "anaphylactin" for the anaphylactic antibody.

Gay and Southard (23), however, who first used this word to designate a "rest" or remain of the antigen, have objected to its use as the designation of an antibody.

While we regret to add another term to the already confusing terminology of anaphylaxis, yet for the sake of brevity one of us has suggested the name "allergin" (1) for the anaphylactic antibody, and we have so used the term in this paper. In using the word "allergin" we do not attempt to define it more definitely than as an antibody characteristic of anaphylaxis, specific for its antigen, and which we regard as the essential agent in the passive transference of anaphylaxis.

TECHNIQUE.

The normal guinea pigs used in all our experiments on passive sensitization were between 200 and 260 grams weight.

The sensitive serum used was always obtained by bleeding sensitive guinea pigs from the neck, defibrinating and centrifuging. It was used as fresh as possible, only in exceptional instances being kept over night in the ice box. The standard amount used was 3 c. c., as this amount proved quite uniformly sufficient to render a normal 250-gram guinea pig quite sensitive within 24 to 48 hours, while smaller amounts (1 to 2 c. c.) often proved ineffective.

The method of administering the sensitive serum was usually by intraperitoneal injection, this being preferable to subcutaneous injection by reason of the quicker absorption.

The interval between the injection of sensitive serum and injection of the test dose of horse serum was at first varied, sometimes 24 and sometimes 48 hours. Subsequently, we adopted a uniform interval of 24 hours after intraperitoneal injection and 48 hours after subcutaneous injection of sensitive serum.

In the great majority of cases hypersusceptibility was tested by injection of 5 c. c. of normal horse serum intraperitoneally, the results obtained by this method proving, on the whole, more satisfactory and more uniform than after intracerebral injection.

NEUTRALIZATION OF ALLERGIN IN SENSITIVE GUINEA PIG AND RABBIT SERUM BY THE SPECIFIC ANTIGEN.

The work of Doerr and Russ (12) on the neutralization of allergin in the serum of rabbits treated by repeated injections of serum has been previously referred to.

Biedl and Kraus (9) found that when normal guinea pigs were given serum from sensitive guinea pigs, followed soon after by the injection of the specific antigen serum, passive transfer of anaphylaxis was prevented. They found the same also true when the order of the injections was reversed.

In the following work, some of which was done before the work of the above authors had come to our notice, we found that allergin in the serum of guinea pigs sensitized by a single injection could be neutralized by admixture, in the test tube, with varying amounts of the specific serum. These experiments were varied in several particulars. Some of them, as will be seen from the tables, were made by mixing the serum of guinea pigs sensitized to horse serum with varying amounts of horse serum, allowing the mixture to remain at room temperature for one hour and then injecting into a guinea pig. The susceptibility of the animal was then tested within 24 to 48 hours by the injection of 5 c. c. of normal horse serum intraperitoneally. In other cases the mixture was allowed to remain at 37° C. for one hour. In still other cases the mixture was injected immediately after mixing.

Some of the experiments bring out very clearly the specificity of the anaphylactic reaction, a point which has been insisted upon by Rosenau and Anderson (39, 40).

TABLE 1.—*Neutralization of allergin by its antigen (horse serum).*

Guinea pigs 12096 and 12097, sensitized 175 days previously with toxin-antitoxin mixture, were bled; blood defibrinated and centrifuged; serum obtained used as follows:

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
12096A	*Control:* 3.5 c. c. sensitive guinea pig serum (g. p. 12096), intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	Very severe symptoms.
12096B	7 c. c. mixture of equal parts of sensitive guinea pig serum and normal horse serum, intraperitoneally, immediately on mixing.	1	.....do................	No symptoms.
12097A	*Control:* 2 c. c. sensitive guinea pig serum (g. p. 12097), intraperitoneally.	1	.....do................	Severe symptoms.
12097B	4 c. c. mixture of equal parts of sensitive guinea pig serum and normal horse serum, intraperitoneally, immediately on mixing.	1	.....do................	No symptoms.

Table 2.—*Absorption of allergin by its antigen (horse serum) mixed and injected at once.*

Eight guinea pigs, sensitized 91 days previously with toxin-antitoxin mixture, were bled; blood defibrinated, centrifuged, and serum used as follows:

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
116	*Control:* 3 c. c. sensitive guinea pig serum, intraperitoneally.	2	5 c. c. normal horse serum, intraperitoneally.	Very severe symptoms.
117	*Control:* Do	2	.....do	Severe symptoms.
118	*Control:* Do	2	.....do	Severe symptoms.
119	3 c. c. sensitive guinea pig serum mixed with 1 c. c. normal horse serum, intraperitoneally, immediately on mixing.	2	.....do	No symptoms.
120	3 c. c. sensitive guinea pig serum mixed with 2 c. c. normal horse serum, intraperitoneally, immediately on mixing.	2	.....do	No symptoms.
121	3 c. c. sensitive guinea pig serum mixed with 4 c.c. normal horse serum, intraperitoneally, immediately on mixing.	2	.....do	No symptoms.
122	3 c.c. sensitive guinea pig serum mixed with 6 c. c. normal horse serum, intraperitoneally, immediately on mixing.	2	.....do	No symptoms.

Table 2a.—*Allergin and antigen (horse serum), 1 hour at 37° C. after mixing.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
123	3 c. c. sensitive guinea pig serum mixed with 1 c. c. normal horse serum, and kept at 37° C. for 1 hour, intraperitoneally.	2	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
124	3 c. c. sensitive guinea pig serum mixed with 2 c. c. normal horse serum, and kept at 37° C. for 1 hour, intraperitoneally.	2	.....do	No symptoms.
125	3 c. c. sensitive guinea pig serum mixed with 4 c. c. normal horse serum, and kept at 37° C. for 1 hour, intraperitoneally.	2	.....do	No symptoms.
126	3 c. c. sensitive guinea pig serum mixed with 6 c. c. normal horse serum, and kept at 37° C. for 1 hour, intraperitoneally.	2	.....do	No symptoms.

Table 3.—*Absorption of allergin by its antigen (horse serum) 1 hour at room temperature after mixing.*

Four guinea pigs, which had been sensitized with a mixture of toxin and antitoxin 240 days previously, were bled; blood defibrinated, centrifuged, and serum used as follows:

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
127	3 c. c. sensitive guinea pig serum mixed with 1 c. c. normal horse serum, kept 1 hour at room temperature, intraperitoneally.	2	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
128	3 c. c. sensitive guinea pig serum mixed with 0.5 c. c. normal horse serum, kept 1 hour at room temperature, intraperitoneally.	2	.....do	No symptoms.
129	3 c. c. sensitive guinea pig serum mixed with 0.25 c. c. normal horse serum, kept 1 hour at room temperature, intraperitoneally.	2	.....do	No symptoms.
130	3 c. c. sensitive guinea pig serum mixed with 0.1 c. c. normal horse serum, kept 1 hour at room temperature, intraperitoneally.	2	.....do	No symptoms.
131	3 c. c. sensitive guinea pig serum mixed with 0.05 c.[c. normal horse serum, kept 1 hour at room temperature, intraperitoneally.	2	.....do	No symptoms.
132	3 c. c. sensitive guinea pig serum mixed with 0.025 c. c. normal horse serum, kept 1 hour at room temperature, intraperitoneally.	2	.....do	No symptoms.

TABLE 3.—*Absorption of allergin by its antigen (horse serum) 1 hour at room temperature after mixing*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
133	3 c. c. sensitive guinea pig serum, mixed with 0.01 c. c. normal horse serum, kept 1 hour at room temperature, intraperitoneally.	2	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
134	*Control:* 3 c. c. sensitive guinea pig serum, intraperitoneally.	2	.....do................	Dead in 30 minutes.
135	*Control:* Do...........................	2	.....do................	Very severe symptoms.

TABLE 4.—*Absorption of allergin by its antigen (horse serum) 1 hour at room temperature.*

Eight guinea pigs, sensitized with toxin-antitoxin mixture 93 days previously, were bled; blood defibrinated, centrifuged, and serum used, as follows:

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
136	3 c. c. sensitive guinea pig serum mixed with 0.01 c. c. normal horse serum, kept 1 hour at room temperature, subcutaneously.	2	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
137	3 c. c. sensitive guinea pig serum mixed with 0.005 c. c. normal horse serum, kept 1 hour at room temperature, subcutaneously.	2	.....do................	No symptoms.
138	3 c. c. sensitive guinea pig serum mixed with 0.001 c. c. normal horse serum, kept 1 hour at room temperature, subcutaneously.	2	.....do................	Very severe symptoms.
139	3 c. c. sensitive guinea pig serum mixed with 0.0005 c. c. normal horse serum, kept 1 hour at room temperature, subcutaneously.	2	.....do................	Severe symptoms.
140	3 c. c. sensitive guinea pig serum mixed with 0.0001 c. c. normal horse serum, kept 1 hour at room temperature, subcutaneously.	2	.....do................	Very severe symptoms.

For *controls*, see below, G. P. Nos. 153 and 154.

TABLE 5.—*Absorption of allergin by its antigen (horse serum) injected separately but simultaneously.*

The following guinea pigs were each given 3 c. c. serum subcutaneously in the right flank and the amounts of normal horse serum indicated in the left flank immediately afterwards. The sensitive serum was obtained from guinea pigs sensitized 93 days previously with a toxin-antitoxin mixture.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
146	3 c. c. sensitive guinea pig serum; 0.01 c. c. normal horse serum; subcutaneously on opposite sides.	2	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
147	3 c. c. sensitive guinea pig serum; 0.005 c. c. normal horse serum; subcutaneously on opposite sides.	2	.....do................	No symptoms.
148	3 c. c. sensitive guinea pig serum; 0.001 c. c. normal horse serum; subcutaneously on opposite sides.	2	.....do................	No symptoms.
149	3 c. c. sensitive guinea pig serum; 0.0005 c. c. normal horse serum; subcutaneously on opposite sides.	2	.....do................	No symptoms.
150	3 c. c. sensitive guinea pig serum; 0.0001 c. c. normal horse serum; subcutaneously on opposite sides.	2	.....do................	Very severe symptoms.
153	*Control:* 3 c. c. sensitive guinea pig serum + 1 c. c. salt solution kept 1 hour at room temperature, subcutaneously.	2	.....do................	Very severe symptoms.
154	*Control:* Do	2	.....do................	Very severe symptoms.

TABLE 6.—*Absorption of horse serum allergin by its antigen (horse serum) and by egg-white.*

Four guinea pigs which had been sensitized 104 days previously with 0.01 c. c. normal horse serum were bled; blood defibrinated, centrifuged, and serum used as follows:

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
166	3 c. c. sensitive guinea pig serum mixed with 0.01 c. c. normal horse serum, subcutaneously, immediately after mixing.	2	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
167	3 c. c. sensitive guinea pig serum mixed with 0.005 c. c. normal horse serum, subcutaneously, immediately after mixing.	2	.....do................	No symptoms.
168	3 c. c. sensitive guinea pig serum mixed with 0.001 c. c. normal horse serum, subcutaneously, immediately after mixing.	2	....do................	Mild symptoms.
169	3 c. c. sensitive guinea pig serum mixed with 0.0005 c. c. normal horse serum, subcutaneously, immediately after mixing.	2	.....do................	Severe symptoms.
170	3 c. c. sensitive guinea pig serum mixed with 0.0001 c. c. normal horse serum, subcutaneously, immediately after mixing.	2	.....do................	Very severe symptoms.
173	3 c. c. sensitive guinea pig serum mixed with 0.001 gram purified egg-white, subcutaneously, immediately after mixing.	2	.....do................	Very severe symptoms.
171	*Control:* 3 c. c. sensitive guinea pig serum, subcutaneously.	2	.....do................	Very severe symptoms.
172	*Control:* Do..........................	2	.....do................	Very severe symptoms.

TABLE 7.—*Neutralization of allergin in sensitive rabbit serum.*

Rabbit No. 4, which had received 5 c. c. normal horse serum 26 days previously, bled; blood defibrinated, centrifuged, and serum used as follows:

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
174	3 c. c. above rabbit serum + 0.01 c. c. normal horse serum, subcutaneously, immediately after mixing.	2	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
175	3 c. c. above rabbit serum + 0.005 c. c. normal horse serum, subcutaneously, immediately after mixing.	2	.....do................	No symptoms.
176	3 c. c. above rabbit serum + 0.001 c. c. normal horse serum, subcutaneously, immediately after mixing.	2	.....do................	Mild symptoms.
177	3 c. c. above rabbit serum + 0.0005 c. c. normal horse serum, subcutaneously, immediately after mixing.	2	.....do................	Marked symptoms.
178	3 c. c. above rabbit serum + 0.0001 c. c. normal horse serum, subcutaneously, immediately after mixing.	2	.....do................	Slight symptoms.
179	*Control:* 3 c. c. above rabbit serum + 1 c. c. normal salt solution, subcutaneously.	2	.....do................	Mild symptoms.
180	*Control:* Do..........................	2	.....do................	Marked symptoms.

TABLE 8.—*Neutralization of egg-white allergin by egg-white.*

Two guinea pigs sensitized 418 days previously with 0.01 c. c. of 5 per cent solution of purified egg-white, subcutaneously, were bled and their mixed serum used as follows:

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
262	3 c. c. sensitive guinea pig serum +0.001 gm. pure egg-white, intraperitoneally.	2	5 c. c. saturated solution raw egg-white, intraperitoneally.	No symptoms.
263	3 c. c. sensitive guinea pig serum + 0.0001 gm. pure egg-white, intraperitoneally.	2	.....do................	No symptoms.
264	3 c. c. sensitive guinea pig serum + 0.000001 gm. pure egg-white, intraperitoneally.	2	.....do................	No symptoms.
265	3 c. c. sensitive guinea pig serum + 1 c. c. normal horse serum, intraperitoneally.	2	.....do................	Severe symptoms.
266	*Control:* 3 c. c. sensitive guinea pig serum, intraperitoneally.	2	.....do................	Severe symptoms.
267	*Control:* do...........................	2	.....do................	Very severe symptoms.

The least amount of horse serum required in any of the foregoing series to prevent passive sensitization of a fresh guinea pig within 24 to 48 hours by 3 c. c. of sensitive guinea pig serum was 0.0005 c. c. In other series 0.001 to 0.005 c. c. of horse serum to 3 c. c. of sensitive serum was required; in all cases 0.01 c. c. proved sufficient. The same effect was obtained whether the horse serum and sensitive serum were mixed and allowed to stand one hour *in vitro* before injection, injected as soon as mixed, or injected separately on different sides of the body at the same time.

Horse serum, as shown above, has no effect in preventing passive sensitization to egg-white; whereas 0.000001 gram of pure egg-white, injected along with serum containing egg-white allergin, is sufficient to neutralize the latter.

The experiments are not sufficient to allow of any conclusions as to whether previous admixture *in vitro* makes a *quantitative* difference in the neutralizing effect of the horse serum. It is rather striking that in two series (Tables 6 and 7), in one of which we used the serum of guinea pigs sensitized with a single injection of horse serum while in the other we used the serum of a rabbit sensitized also with a single (much larger) injection, the quantitative relations of sensitive serum to horse serum are the same.

EFFECT OF INJECTIONS OF ANTIGEN AND ALLERGIN SEPARATED BY VARIOUS INTERVALS.

In order to determine the effect on passive sensitization of the injection of antigen and allergin separated by various intervals two series of experiments were done.

In the first series of experiments normal guinea pigs were given a definite amount (0.01 c. c.) of horse serum subcutaneously, and at various intervals thereafter, up to 48 hours, 3 c. c. of sensitive guinea pig serum.

In the second series normal guinea pigs were first given 3 c. c. of sensitive guinea pig serum, and at various intervals, up to 24 hours, were given 0.01 c. c. of normal horse serum, both injections being made subcutaneously on opposite sides of the body.

Hypersusceptibility was tested 48 hours after the injection of the sensitive serum (therefore 24 to 48 hours after injection of the horse serum—long enough for at least the greater part of this to have been absorbed).

TABLE 9.—*Absorption of allergin by its antigen (horse serum) injected previously (6 to 48 hours).*

All of the guinea pigs of this series received, at 9 a. m. on March 14, 1910, 0.01 c. c. normal horse serum subcutaneously; and at intervals noted received serum of sensitive guinea pigs as follows:

Serum 1, is the mixed serum of 12 guinea pigs which had received 0.01 c. c. normal horse serum 107 days previously; bled March 14.

Serum 2, is the mixed serum of sensitive guinea pigs of this same lot, bled March 15.

Serum 3, is the mixed serum of sensitive guinea pigs of this same lot, bled March 16.

The sensitive guinea pigs were bled on different dates in order to avoid deterioration of the serum by keeping.

Controls, as will be noted, were made with each lot of serum.

Passive sensitization was tested in each case between 36 and 48 hours after receiving the sensitive serum.

Normal G. P. No.	First injection.	Interval in days.	Second injection.	Result.
181	*Control:* 3 c. c. sensitive guinea pig serum 1, subcutaneously, simultaneously with horse serum.	2	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
182	*Control:* do	2	do	No symptoms.
183	3 c. c. sensitive guinea pig serum 1, subcutaneously, 6 hours later.	2	do	No symptoms.
184	do	2	do	No symptoms.
185	3 c. c. sensitive guinea pig serum 1, subcutaneously, 12 hours later.	2	do	No symptoms.
186	do	2	do	No symptoms.
187	3 c. c. sensitive guinea pig serum 1, subcutaneously, 15 hours later.	2	do	No symptoms.
188	do	2	do	Slight symptoms.
189	3 c. c. sensitive guinea pig serum 2, subcutaneously, 24 hours later.	2	do	Slight symptoms.
190	do	2	do	Mild symptoms.
191	3 c. c. sensitive guinea pig serum 2, subcutaneously, 30 hours later.	2	do	Slight symptoms.
192	do	2	do	Slight symptoms.
193	3 c. c. sensitive guinea pig serum 2, subcutaneously, 36 hours later.	2	do	Slight symptoms.
194	do	2	do	Mild symptoms.
195	3 c. c. sensitive guinea pig serum 2, subcutaneously, 39 hours later.	2	do	Slight symptoms.
196	do	2	do	Slight symptoms.
197	3 c. c. sensitive guinea pig serum 3, subcutaneously, 48 hours later.	2	do	Mild symptoms.
198	do	2	do	Slight symptoms.
201	*Control:* 3 c. c. sensitive guinea pig serum 1, intraperitoneally.	2	do	Marked symptoms.
202	*Control:* do	2	do	Marked symptoms.
203	*Control:* 3 c. c. sensitive guinea pig serum 2, intraperitoneally.	2	do	Very severe symptoms.
204	*Control:* do	2	do	Severe symptoms.
205	*Control:* 3 c. c. sensitive guinea pig serum 3, intraperitoneally.	2	do	Marked symptoms.
206	*Control:* do	2	do	Marked symptoms.

The results in this series show that 0.01 c. c. of horse serum constantly prevents passive sensitization with 3 c. c. sensitive guinea pig serum, provided the latter is given *within 12 hours* after the horse serum. If the interval is longer (15 to 48 hours) passive hypersusceptibility is modified, but not constantly prevented.

We are led to infer from this that this amount of horse serum (0.01 c. c.) within 12 to 48 hours after its injection into the body of a normal guinea pig has undergone such changes (probably at least the preliminary steps toward assimilation) that the amount of horse serum as such present in the circulation is appreciably diminished, since it has already been demonstrated that from 0.005 to 0.001 c. c. of horse serum present in the circulation at the time of injection of allergin is sufficient to prevent passive sensitization.

TABLE 10.—*Absorption of allergin by its antigen (horse serum) injected subsequently (3 to 24 hours).*

All the following guinea pigs received 3 c. c. sensitive guinea pig serum and at the intervals noted received 0.01 c. c. normal horse serum.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
181	3 c. c. sensitive guinea pig serum 1; at the same time, 0.01 c. c. normal horse serum, subcutaneously.	2	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
192	do	2	do	No symptoms.
207	3 c. c. sensitive guinea pig serum 1; 3 hours later, 0.01 c. c. normal horse serum, subcutaneously.	2	do	No symptoms.
208	do	2	do	No symptoms.
209	3 c. c. sensitive guinea pig serum 1; 6 hours later, 0.01 c. c. normal horse serum, subcutaneously.	2	do	No symptoms.
210	do	2	do	Mild symptoms.
211	3 c. c. sensitive guinea pig serum 1; 12 hours later, 0.01 c. c. normal horse serum, subcutaneously.	2	do	No symptoms.
212	do	2	do	Slight symptoms.
213	3 c. c. sensitive guinea pig serum 1; 15 hours later, 0.01 c. c. normal horse serum, subcutaneously.	2	do	Slight symptoms.
214	do	2	do	No symptoms.
215	3 c. c. sensitive guinea pig serum 1; 24 hours later, 0.01 c. c. normal horse serum, subcutaneously.	2	do	Slight symptoms.
216	do	2	do	Mild symptoms.
217	*Control:* 3 c. c. sensitive guinea pig serum 1, subcutaneously.	2	do	Severe symptoms.
218	*Control:* do	2	do	Severe symptoms.

Passive anaphylaxis was prevented in those guinea pigs which received the horse serum *within 6 hours* after receiving the sensitive guinea pig serum. In the remainder of the series, passive anaphylaxis was either prevented or modified.

It has been previously shown that a guinea pig becomes sensitive within 24 hours after injection of sensitive serum, the gradual development of sensitiveness being generally ascribed to a slow binding of the allergin. Our results indicate that within 6 hours the allergin has become "bound" in some way, so that it can no longer be neutralized by the injection of horse serum in the amount used by us—an amount considerably exceeding that necessary to prevent passive sensitization when injected simultaneously with the allergin.

SPECIFICITY OF NEUTRALIZATION OF ALLERGIN.

A series of guinea pigs were injected intraperitoneally each with 3 c. c. mixed serum of 16 guinea pigs which had been sensitized to *horse serum* 60 days to 90 days previously. To this sensitive serum was added normal serum of various animals in amounts shown in the table. The mixture was allowed to stand 1 hour at room temperature before injection. Susceptibility was tested after 24 hours by injection of 5 c. c. of *horse serum* intraperitoneally.

TABLE 11.—*Specificity of neutralization of allergin.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	3 c. c. sensitive guinea pig serum:			
386	+1 c. c. human serum	1	5 c. c. normal horse serum, intraperitoneally.	Marked symptoms.
391	+1 c. c. rabbit serum	1	do	Marked symptoms.
385	+1 c. c. dog serum	1	do	Marked symptoms.
384	+1 c. c. cat serum	1	do	Marked symptoms.
392	+1 c. c. chicken serum	1	do	Marked symptoms.
389	+1 c. c. ox serum	1	do	Marked symptoms.
387	+1 c. c. goat serum	1	do	Mild symptoms.
387A	+0.1 c. c. goat serum	1	do	Marked symptoms.
388	+1 c. c. sheep serum	1	do	Mild symptoms.
388A	+0.1 c. c. sheep serum	1	do	Marked symptoms.
388B	+0.01 c. c. sheep serum	1	do	Marked symptoms.
390	+1 c. c. hog serum	1	do	Mild symptoms.
390A	+0.1 c. c. hog serum	1	do	Marked symptoms.
390B	+0.01 hog serum	1	do	Marked symptoms.
379	+1 c. c. donkey serum	1	do	*No symptoms.*
380	+0.1 c. c. donkey serum	1	do	*No symptoms.*
381	+0.01 c. c. donkey serum	1	do	Marked symptoms.
382	+0.005 c. c. donkey serum	1	do	Marked symptoms.
383	+0.001 c. c. donkey serum	1	do	Marked symptoms.
375	+0.01 c. c. horse serum	1	do	*No symptoms.*
376	+0.005 c. c. horse serum	1	do	*No symptoms.*
377	+0.001 c. c. horse serum	1	do	*No symptoms.*
378	+0.0005 c. c. horse serum	1	do	Mild symptoms.
393	+1 c. c. milk	1	do	Marked symptoms.
373	*Control:* 3 c. c. sensitive guinea pig serum	1	do	Marked symptoms.
374	*Control:* do	1	do	Marked symptoms.

The foregoing experiment shows clearly the specificity of this action of antigen in neutralizing the passive sensitizing effect of sensitive guinea pig serum. While 0.001 c. c. of the specific antigen, horse serum, was sufficient to prevent passive sensitization, it required 0.1 c. c. of donkey serum to produce this effect; 1 c. c. of goat serum, sheep serum, and hog serum caused doubtful diminution of the hypersusceptibility; 0.1 c. c. of each of these three sera caused no diminution. Of the other sera (six) and of milk, 1 c. c. caused no diminution in the hypersusceptibility as compared to the controls.

The effect of horse serum, then, as compared to donkey serum, is as 100:1; as compared to the other sera, at least 1,000:1.

The fact that such minute quantities as 0.001 c. c. of horse serum, or 0.000.001 gram of pure egg-white will neutralize the passive sensitizing effect of their respective antisera, calls attention to the necessity of exercising the utmost care in performing experiments with sensitive serum. A syringe, for instance, which has been used for injecting horse serum may, after even careful washing out, or ordinary "surgical" sterilization, contain a trace of serum sufficient to cause serious error if this same syringe is used subsequently for injecting sensitive serum.

CAN GUINEA PIGS BE ACTIVELY SENSITIZED BY MIXTURES OF HORSE SERUM AND ALLERGIN?

Having determined that 0.005 c. c. to 0.0005 c. c. of horse serum will prevent *passive* sensitization of a normal guinea pig within 24 to 48 hours, experiments were made to determine whether by such a mixture guinea pigs could be *actively* sensitized.

A series of guinea pigs were injected with 3 c. c. of sensitive guinea pig serum, plus varying amounts from 0.01 c. c. to 0.0001 c. c. of horse serum (the sensitive serum used was the same lot of serum as used in series 146–154, Table 5).

After an incubation period of *19 days* these guinea pigs were tested for hypersusceptibility.

TABLE 12. *Active sensitization by mixtures of allergin and antigen (horse serum).*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
141	3 c. c. sensitive guinea pig serum, mixed with 0.01 c. c. normal horse serum, kept 1 hour at room temperature, subcutaneously.	19	5 c. c. normal horse serum, intraperitoneally.	Very severe symptoms.
142	3 c. c. sensitive guinea pig serum, mixed with 0.005 c. c. normal horse serum, kept 1 hour at room temperature, subcutaneously.	19	.....do................	Very severe symptoms.
143	3 c. c. sensitive guinea pig serum, mixed with 0.001 c. c. normal horse serum, kept 1 hour at room temperature, subcutaneously.	19	.....do................	Severe symptoms.
144	3 c. c. sensitive guinea pig serum, mixed with 0.0005 c. c. normal horse serum, kept 1 hour at room temperature, subcutaneously.	19	.....do................	Very severe symptoms.
145	3 c. c. sensitive guinea pig serum, mixed with 0.0001 c. c. normal horse serum, kept 1 hour at room temperature, subcutaneously.	19	.....do................	Very severe symptoms.
151	*Control:* 3 c. c. *normal guinea pig serum*, mixed with 0.01 c. c. normal horse serum, kept 1 hour at room temperature, subcutaneously.	19	.....do................	Very severe symptoms.
152	*Control:* 3 c. c. *normal guinea pig serum*, mixed with 0.0001 c. c. normal horse serum, kept 1 hour at room temperature, subcutaneously.	19	.....do................	No symptoms.

As was to be expected, all the animals which had received the mixtures of horse serum and sensitive guinea pig serum were found, in this case, to be *sensitive*. In those that received an *excess* of horse serum this is capable of explanation by assuming that the excess of horse serum actively sensitized them. In those that received an amount of horse serum *less* than necessary to completely saturate the allergin, the excess of the latter may have been the agent in sensitizing. This may explain the sensitization of guinea pig No. 145, whose control, G. P. No. 152, which received the *same amount* of horse serum and *normal* guinea pig serum, did not become sensitive. But while, in all probability, none of the mixtures was exactly "balanced," it seems probable that even a perfectly balanced mixture would cause sensitization, given a sufficient incubation, considering the fact that balanced toxin-antitoxin mixtures stimulate the organism to further formation of antitoxin (antibody).

ABSORPTION OF ALLERGIN BY ORGANS OF NORMAL AND SENSITIVE GUINEA PIGS.

According to Friedberger's (15) theory of anaphylaxis, it is necessary for passively transferred antibody to become fixed to the tissue cells in order to render the animal sensitive.

Doerr and Russ (12), in support of this view, cite a single series of experiments in which they added to sensitive rabbit serum normal guinea-pig tissues, separated the serum by centrifuging after 4 hours' contact, and found that in certain cases the serum so treated failed to passively sensitize normal guinea pigs. They present this observation as a support to the view that anaphylactic antibody is bound to the tissue cells *in vivo;* but in view of their small number of experiments these authors themselves point out that such a conclusion is not yet justified.

In an attempt to confirm their results we made two series of experiments, using in one the organs of *normal* guinea pigs, in the other the corresponding organs of *sensitive* guinea pigs. The same lot of sensitive serum was used in both series. The conditions of our experiment vary from those of Doerr and Russ in that we used sensitive *guinea-pig* serum while they used sensitive (precipitating) serum from a *rabbit;* we added 2 grams of tissue to 6 c. c. of sensitive serum, while they used 1 gram to 3.5 c. c.

TABLE 13.—*Absorption of allergin by organs of normal guinea pigs.*

Blood of 16 sensitive guinea pigs was mixed and defibrinated; serum used as follows:

Two grams of each of the organs named from normal guinea pigs were ground in a mortar; 6 c. c. of sensitive guinea-pig serum added, kept at 37° C. 4 hours in a moist chamber; centrifuged; supernatant fluid removed and used as follows:

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
304	3 c. c. supernatant fluid from mixture of sensitive guinea-pig serum and normal guinea-pig *brain*, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	Marked symptoms.
305	3 c. c. supernatant fluid from mixture of sensitive guinea-pig serum and normal guinea-pig *heart*, intraperitoneally.	1	.....do................	Severe symptoms.
306	3 c. c. supernatant fluid from mixture of sensitive guinea-pig serum and normal guinea-pig *liver*, intraperitoneally.	1	.....do................	Marked symptoms.
307	3 c. c. supernatant fluid from mixture of sensitive guinea-pig serum and normal guinea-pig *spleen*, intraperitoneally.	1	.....do................	Severe symptoms.
308	3 c. c. supernatant fluid from mixture of sensitive guinea-pig serum and normal guinea-pig *kidney*, intraperitoneally.	1	.....do................	Severe symptoms.
309	3 c. c. supernatant fluid from mixture of sensitive guinea-pig serum and normal guinea-pig *small intestine*, intraperitoneally.	1	.....do................	Marked symptoms.
310	3 c. c. supernatant fluid from mixture of sensitive guinea-pig serum and normal guinea-pig *femoral muscle*, intraperitoneally.	1	.....do................	Severe symptoms.
311	3 c. c. supernatant fluid from mixture of sensitive guinea-pig serum and normal guinea-pig *corpuscles* washed six times (equal volume of washed corpuscles and sensitive guinea-pig serum), intraperitoneally.	1	.....do................	Marked symptoms.
295	*Control:* 3 c. c. sensitive guinea-pig serum kept 4 hours at 37° C. intraperitoneally.	1	.....do................	Severe symptoms.
296	*Control:* Do..........................	1	.....do................	Very severe symptoms.

TABLE 14.—*Absorption of allergin by organs of sensitive guinea pigs.*

Sensitive guinea pig serum the same as used in the preceding series.

Organs obtained from three guinea pigs, sensitized 63 days previously with 0.01 c. c. normal horse serum.

Six c. c. of the sensitive serum added to 2 grams of each of the organs named; ground in mortar; kept 4 hours at 37° C.; centrifuged; 3 c. c. of the supernatant fluid injected into normal guinea pigs.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
297	3 c. c. supernatant fluid from mixture of sensitive guinea pig serum and sensitive guinea pig *brain*, intraperitoneally	1	5 c. c. normal horse serum, intraperitoneally.	Very severe symptoms.
298	3 c. c. supernatant fluid from mixture of sensitive guinea pig serum and sensitive guinea pig *heart*, intraperitoneally.	1	.....do................	Severe symptoms.
299	3 c. c. supernatant fluid from mixture of sensitive guinea pig serum and sensitive guinea pig *liver*, intraperitoneally.	1	.....do................	Marked symptoms.
300	3 c. c. supernatant fluid from mixture of sensitive guinea pig serum and sensitive guinea pig *spleen*, intraperitoneally.	1	.....do................	Marked symptoms.
301	3 c. c. supernatant fluid from mixture of sensitive guinea pig serum and sensitive guinea pig *kidney*, intraperitoneally.	1	.....do................	Severe symptoms.
302	3 c. c. supernatant fluid from mixture of sensitive guinea pig serum and sensitive guinea pig *small intestine*, intraperitoneally.	1	.....do................	Severe symptoms.
303	3 c. c. supernatant fluid from mixture of sensitive guinea pig serum and sensitive guinea pig *femoral muscle*, intraperitoneally.	1	.....do................	Marked symptoms.

It is seen from the above experiments (Tables 13 and 14) that the anaphylactic antibody was *not* removed from sensitive guinea pig serum by 4-hour contact with various organs of normal or sensitive guinea pigs, nor was the amount of antibody apparently diminished by this procedure.

The results of our experiments, therefore, fail to confirm the findings of Doerr and Russ, although we believe, after careful comparison, that the conditions of our experiment were even more favorable to obtaining a positive result of fixation than were the conditions of Doerr and Russ's experiments.[a]

Giving due weight to quantitative considerations and the artificial conditions of an experiment *in vitro*, we can not take these experiments as proving that binding of anaphylactic antibody by normal guinea pig tissues does not take place in the living organism.

DO THE TISSUES OF SENSITIVE GUINEA PIGS CONTAIN ALLERGIN?

It has been shown by the work of Schultz (44), already referred to, that the smooth muscle tissue of a sensitive guinea pig reacts specifically toward the sensitizing antigen (horse serum). It had been assumed, independently of this result, by Friedberger (15) and others that the tissues of sensitive animals contain "sessile" or fixed anaphylactic antibody. It occurred to us, therefore, to determine whether a normal guinea pig could be passively sensitized by injecting an emulsion of the *tissues* of a sensitive guinea pig.

TABLE 15.—*Do the tissues of sensitive guinea pigs contain allergin?*

About 3 or 4 grams each of kidney and brain from three sensitive guinea pigs, which were killed by bleeding, were ground up with sand; 10 c. c. normal salt solution added, making a thick emulsion; sedimented in test glasses to remove the sand and coarse particles; injected into normal guinea pigs.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
290	10 c. c. emulsion of sensitive guinea pig kidney, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
291	10 c. c. emulsion of sensitive guinea pig brain, intraperitoneally.	1	.....do..............	No symptoms.
155	5 c. c. washed corpuscles, washed four times, obtained from sensitive guinea pig, subcutaneously.	2	.....do.................	No symptoms.

Guinea pigs Nos. 295 and 296, Table 13, received serum of the sensitive guinea pigs whose emulsified organs were injected into guinea pigs Nos. 290 and 291.

Guinea pigs Nos. 153 and 154, Table 5, received *serum* from which were separated the corpuscles injected into guinea pig No. 155.

We have been unable to passively sensitize guinea pigs by injecting an emulsion of the brain or kidney of a sensitive guinea pig or by injecting the washed corpuscles of a sensitive guinea pig. Braun (10) has recently reported similar experiments, likewise with purely negative results.

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[a] Doerr and Russ, in subsequent publications, have not mentioned any further experiments in this line, nor have their results in this case been confirmed by any other observer so far as we have been able to ascertain.

While these negative results certainly fail to give any evidence of allergin existing in the blood corpuscles and tissues of sensitive guinea pigs, they do not exclude the possibility of the existence of such "fixed" antibody, for "sessile" antibody is conceived to be an integral part of the cell, from which it is normally dissociated only by the *vital activities* of the living organism.

ALLERGIN IN SERUM OF GUINEA PIGS AT VARIOUS INTERVALS AFTER RECOVERY FROM ANAPHYLACTIC SHOCK.

Many workers have shown that the serum of immune guinea pigs contains allergin, but we are not aware of any work other than that of Rosenau and Anderson (41) having been reported as to how soon after recovery from a second large injection of serum allergin may be demonstrated in the serum of guinea pigs. The following work was designed to show how soon after recovery from a massive injection of serum allergin can be demonstrated in the serum of such animals.

TABLE 16.—*Allergin in the serum of guinea pigs moribund from anaphylactic shock.*

Five c. c. normal horse serum was injected intraperitoneally into each of two sensitive guinea pigs These animals, when moribund, were bled, the blood defibrinated and centrifuged, and the serum used as follows:

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
233	3 c. c. above guinea pig serum, subcutaneously.	2	0.1 c. c. normal horse serum, into brain.	No symptoms.
234	.do......	2	.....do................	No symptoms.

TABLE 17.—*Allergin in the serum of guinea pigs recently (5 to 72 hours) recovered from anaphylactic shock.*

In this series were used 8 guinea pigs sensitized 62 days previously with 0.01 c. c. normal horse serum subcutaneously. Two of these were bled, and their serum used to passively sensitize two normal guinea pigs (controls). The remainder of the sensitized guinea pigs received each 1 c. c. normal horse serum intraperitoneally. All showed symptoms.

At the intervals indicated in the following tables these guinea pigs were bled, and their serum tested for allergin.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
313 A	*Control:* 3 c. c. serum of sensitive G. P. 313 and 314, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	Marked symptoms.
314 A	*Control:* do......	1	.....do................	Mild symptoms.
315	0.01 c. c. normal horse serum, subcutaneously.	62	1 c. c. normal horse serum, intraperitoneally.	Very severe symptoms.
316	.do......	62	.....do................	Very severe symptoms.
317	.do......	62	.....do................	Very severe symptoms.
318	.....do................................	62	.....do................	Very severe symptoms.
319	.do......	62	.... do................	Dead in 35 minutes.
320	.do......	62	.....do................	Dead in 60 minutes.

TABLE 17.—*Allergin in the serum of guinea pigs recently (5 to 72 hours) recovered from anaphylactic shock*—Continued.

G. P. NOS. 315 AND 316 BLED *5 HOURS* AFTER RECOVERY.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
315 A	3 c. c. serum of recovered G. P. 315 and 316, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
316 A	.....do	1	.....do	No symptoms.

G. P. NOS. 317 AND 318 BLED *3 DAYS* AFTER RECOVERY.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
317 A	3 c. c. serum of recovered G. P. 317 and 318, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
318 A	.....do	1	.....do	No symptoms.

TABLE 18.—*Allergin in serum of guinea pigs, 17 days after recovery from anaphylactic shock.*

Guinea pigs Nos. 272 and 273, bled April 7, 1910. Previous treatment as follows:
November 27, 1909, 0.01 c. c. normal horse serum subcutaneously.
February 8, 1910, 1 c. c. normal horse serum subcutaneously; symptoms.
March 21, 1910, 6 c. c. normal horse serum intraperitoneally; slight symptoms.
April 7, 1910, killed. Serum precipitates horse serum 1:10; negative 1:100 in 20 hours.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
350	3 c. c. serum of G. P. Nos. 272 and 273 mixed, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	Mild symptoms.
351	2 c. c. do	1	.....do	Mild symptoms.
352	1 c. c. do	1	.....do	Symptoms. (?)

TABLE 19.—*Allergin in serum of guinea pigs 41 days after recovery from anaphylactic shock.*

Guinea pigs Nos. 268–273 received 0.01 c. c. normal horse serum. Received 1 c. c. normal horse serum subcutaneously 72 days later. All had symptoms, but recovered.

Nos. 268–271 were killed March 21, 1910, and serum tested for allergin by passive transference. Nos. 272 and 273 tested for active anaphylaxis, as an index of the susceptibility of this lot of guinea pigs. As shown below, these recovered guinea pigs were relatively immune to anaphylactic shock.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
272	*Control:* 0.01 c. c. normal horse serum. 72 days later, 1 c. c. normal horse serum subcutaneously; symptoms, recovered.	41	6 c. c. normal horse serum, intraperitoneally.	Symptoms. (?)
273	*Control:* do	41	.....do	Mild symptoms.
274	3.5 c. c. serum G. P. Nos. 268–271 intraperitoneally.	2	0.1 c. c. normal horse serum, into brain.	Very severe symptoms.
275	.....do	2	.....do	Very severe symptoms.
276	.....do	2	.....do	Severe sypmtoms.
277	.....do	2	.....do	Marked symptoms.

TABLE 20.—*Allergin in serum of a recovered guinea pig 450 days after recovery from anaphylactic shock.*

G. P. No. 353.

January 12, 1906, 0.19 gm. toxin No. 7+1 unit antitoxic horse serum B27.

January 12, 1909, —3 years later. 6 c. c. normal horse serum subcutaneously. Symptoms.

April 7, 1910, 6 c. c. normal horse serum intraperitoneally. Slight symptoms. Bled from heart about 15 c. c. before last test; serum obtained used in the following experiment·

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
353 A	3 c. c. serum G. P. 353 intraperitoneally..	1	5 c. c. normal horse serum, intraperitoneally.	Marked symptoms.
353 B	2 c. c. do..................................	1	.....do.................	Marked symptoms.

Summarizing the foregoing results, it has been shown that guinea pigs, during the later stage of anaphylactic shock and shortly thereafter, do not have in their serum sufficient free allergin to passively sensitize normal guinea pigs. At longer intervals after recovery from anaphylactic shock (17 to 450 days) recovered guinea pigs do contain free allergin in their serum, even when themselves *very slightly* susceptible to anaphylactic shock.

More extended work on this line will be necessary before generalizations are warranted. The few experiments above recorded are, however, at least highly suggestive of saturation of allergin taking place during anaphylactic shock.

QUANTITATIVE ESTIMATION OF ALLERGIN IN THE SERUM OF SENSITIVE GUINEA PIGS, RECOVERED GUINEA PIGS, AND RABBITS TREATED BY REPEATED INJECTIONS OF SERUM.

Braun (10) found that it would take at least 3.5 c. c. of serum from a guinea pig sensitized with a toxin-antitoxin mixture, and 1 c. c. from guinea pigs that had received three injections of 5 c. c. each, to convey passive anaphylaxis within 24 hours.

Doerr and Russ (11, 12), Friedberger (16, 17), and others, working with the serum of rabbits treated with repeated injections of serum, found that amounts much less than 1 c. c. would passively sensitize guinea pigs.

We report below experiments as to the amount of allergin in the serum of guinea pigs sensitized by a single small injection, in the serum of recovered guinea pigs, and in the serum of rabbits, which had been sensitized by repeated injections.

TABLE 21.—*Quantitative estimation of allergin in serum of guinea pigs sensitized with a single injection of horse serum.*

Three guinea pigs which had been sensitized 72 days previously with 0.01 c. c. normal horse serum subcutaneously, were bled, and the serum used as follows:

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
332	3 c. c. above sensitive guinea pig serum, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	Symptoms (?).
332A	3 c. c. do	15	do	Severe symptoms.
333	2 c. c. do	1	do	Symptoms (?).
333A	2 c. c. do	15	do	Marked symptoms.
334	1 c. c. do	1	do	No symptoms.
334A	1 c. c. do	15	do	Mild symptoms.
335	0.5 c. c. do	1	do	No symptoms.
335A	0.5 c. c. do	15	do	No symptoms.
336	0.1 c. c. do	1	do	No symptoms.
336A	0.1 c. c. do	15	do	No symptoms.

TABLE 22.—*Quantitative estimation of allergin in the serum of recovered guinea pigs.*

Five guinea pigs were injected subcutaneously with 0.01 c. c. normal horse serum November 27, 1909.

February 8, 1910, 73 days later, 1 c. c. normal horse serum subcutaneously; symptoms.

April 4=55 days after last injection, killed, and serum tested as follows:

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
327	3 c. c. above sensitive guinea pig serum, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	Very severe symptoms.
328	2 c. c. do	1	do	Severe symptoms.
329	1 c. c. do	1	do	Marked symptoms.
345	1 c. c. do	1	do	Severe symptoms.
330	0.5 c. c. do[a]	1	do	Symptoms (?).
330B	0.5 c. c. do	1	do	Symptoms (?).
346	0.5 c. c. do	1	do	No symptoms.
331	0.1 c. c. do	1	do	Symptoms (?).
347	0.1 c. c. do	1	do	No symptoms.

[a] Part of the injection of 330 was lost; hence, 330B was substituted in this series.

TABLE 23.—*Quantitative estimation of allergin in serum of a rabbit that had received repeated injections of serum.*

Rabbit No. 9, treated as follows:

February 14, 1910, 5 c. c. normal horse serum subcutaneously.

March 16, 7 c. c. normal horse serum intravenously.

March 14, 10 c. c. normal horse serum intravenously.

March 22, 10 c. c. normal horse serum intravenously.

Precipitates normal horse serum 1:1000 in 2 hours.
1:5000 in 24 hours.

Bled April 4. Serum used as below:

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
337	3 c. c. serum of rabbit No. 9, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	Very severe symptoms.
338	2 c. c. do	1	do	Very severe symptoms.
339	1 c. c. do	1	do	Severe symptoms.
348	1 c. c. do	1	do	No symptoms.
340	0.5 c. c. do	1	do	Very severe symptoms.
342	0.5 c. c. do	1	do	Symptoms (?).
343	0.25 c. c. do	1	do	Marked symptoms.
341	0.1 c. c. do	1	do	No symptoms.
344	0.1 c. c. do	1	do	Symptoms (?).

The results in these experiments are somewhat irregular, as results are apt to be when one uses amounts of sensitive serum approaching the minimum, as it is difficult to distinguish anaphylactic symptoms when very slight. Taken in connection with the rest of our work, however, these experiments indicate pretty clearly—

(1) That 3 c. c. of the serum of guinea pigs sensitized (more than 30 days previously) with a single injection of horse serum is approximately the minimum amount that will *constantly* passively sensitize normal 250-gram guinea pigs so that they will react definitely to intraperitoneal injection of 5 c. c. horse serum 24 hours later.

(2) That the serum of "immune" guinea pigs contains very considerably *more* allergin (at least three times as much in the above experiment).

(3) That the serum of a rabbit treated with frequent large injections of horse serum contains more free allergin than the serum of highly sensitive guinea pigs.

EFFECT OF PROLONGED INCUBATION PERIOD ON PASSIVE SENSITIZATION.

Gay and Southard (23, 24), Otto (34), and Lewis (25) have reported that a very small amount of the serum of a sensitive guinea pig is sufficient to passively sensitize a normal guinea pig after an incubation period of 15 days or more.

Rosenau and Anderson (41), on the other hand, found that after subcutaneous injection of sensitive guinea-pig serum, in amounts from 1.5 to 4 c. c., into normal guinea pigs, the latter were not constantly sensitive after intervals of 16 to 26 days. They found, however, that guinea pigs tested 2 days after subcutaneous injection of sensitive guinea-pig serum were quite constantly sensitive.

In order to determine whether a prolonged incubation period actually diminishes or increases passive hypersusceptibility we injected parallel series (see Table 21) of guinea pigs with the same lot of mixed sensitive guinea-pig serum, tested one series after 24 hours and the other series after 15 days. Comparison of the results shows that the degree of hypersusceptibility conferred by equal amounts of sensitive serum was greater after 15 days than after 1 day. Even after 15 days, however, our guinea pigs were not sensitized by less than 1 c. c. of the sensitive serum.

A more extended study of the influence of time on passive sensitization is necessary before the exact influence of this factor can be accurately estimated.

PRODUCTION OF IMMEDIATE ANAPHYLACTIC SHOCK IN NORMAL GUINEA PIGS BY THE INJECTION OF MIXTURES OF ALLERGIN AND ANTIGEN.

Richet (37) has reported the production of anaphylactic symptoms in a normal dog by the intravenous injection of a mixture *in vitro* of the serum from a dog that had recovered about 30 days previously from a second injection of crepitine and an equal amount of a weak solution of crepitine; the injection of an equal amount of serum from an untreated dog and a solution of crepitine produced no symptoms in a normal animal.

Biedl and Kraus (9) caused anaphylactic symptoms in normal guinea-pigs by the intravenous injection of a mixture of sensitive guinea pig serum and horse serum. Doerr and Russ (12) had previously reported similar results from sensitive *rabbit* serum and specific antigen mixed *in vitro*.

TABLE 24.—*Immediate anaphylactic shock in normal guinea pigs, following the injection of a mixture of horse serum and allergin.*

Serum obtained from 5 guinea pigs which had been treated as follows:
0.01 c. c. normal horse serum, subcutaneously.
73 days later, 1 c. c. normal horse serum, subcutaneously; symptoms.
Bled 56 days after last injection.
(Some of this same serum was used to passively sensitize guinea pigs 327 to 331, q. v., p. 27.)

Normal G. P. No.	Injection.	Result.
342A	4 c. c. above guinea pig serum + 1 c. c. normal horse serum; mixed and allowed to stand 45 minutes at room temperature; intraperitoneally.	Slight symptoms.
343A	.....do	Do.
344A	.....do	Do.
345A	.....do	Do.

All these guinea pigs showed rather indefinite symptoms—scratched with fore feet, had muscular twitchings, dyspnœa—but appeared quite sick.

TABLE 25.—*Anaphylactic shock from injection of horse serum and allergin mixture.*

G. P. No. 100 received 0.01 c. c. normal horse serum, subcutaneously.
73 days later, received 1 c. c. normal horse serum, subcutaneously; slight symptoms.
Was bled 13 days later; blood defibrinated, centrifuged and the serum used in the following experiment:

Normal G. P. No.	Injection.	Result.
100A	4 c. c. mixture of equal parts of serum of guinea pig No. 100 and normal horse serum, intraperitoneally.	Slight symptoms within 1 hour.
100B	6 c. c. do	Mild symptoms within 1 hour.

TABLE 26.

Serum from 5 guinea pigs which had been treated as follows:

0.01 c. c. normal horse serum subcutaneously.

73 days later, 1 c. c. normal horse serum subcutaneously.

Bled 56 days later.

(For passive sensitizing effect of this serum, see guinea pigs Nos. 327 to 331, 345 to 347, p. 27.)

4 c. c. of this serum + 1 c. c. of normal horse serum allowed to stand 1 hour at 37° C., then 1 hour at room temperature.

Considerable precipitate, which was well shaken up before injection.

Normal G. P. No.	Injection.	Result.
351	2 c. c. above mixture of "immune" guinea pig serum and normal horse serum, *intravenously*.	Severe anaphylactic symptoms at once. Sick for 2 hours.
350	*Control:* 2 c. c. normal guinea pig serum, *intravenously*.....	No symptoms.

Doerr and Russ (12) showed that when a strong precipitating serum was mixed with its antigen, the resulting precipitate, dissolved in weak soda solution and injected intravenously, caused symptoms of anaphylactic shock in normal guinea pigs, while the supernatant fluid did not.

Friedberger (17) took well washed precipitate, obtained by adding precipitating rabbit serum of high titer to its specific antigen serum, added fresh normal guinea pig serum, left in contact a short while, then separated the precipitate from the serum by centrifuging and found that the normal guinea pig serum so treated, when injected intravenously (2 c. c.) into normal guinea pigs, caused immediate, severe, and sometimes fatal anaphylactic shock. When, instead of fresh guinea pig serum, he used in the same way, physiological salt solution or guinea pig serum inactivated by heating to 60° C., the fluid, after centrifugation, had little or no effect upon normal guinea pigs. He concluded that the *complement* is necessary to the extraction of a toxic substance (anaphyla-toxin) from precipitate. We report below an experiment confirming Friedberger's observations.

TABLE 27. *Anaphylactic shock from normal guinea pig serum treated with washed precipitate.*

Rabbit No. 9 had been treated as follows:
February 14, 1910, 5 c. c. normal horse serum, subcutaneously.
March 16, 7 c. c. normal horse serum, intravenously.
March 19, 10 c. c. normal horse serum, intravenously.
March 22, 10 c. c. normal horse serum, intravenously.

The serum obtained April 4 precipitated normal horse serum in a dilution of 1 : 1,000 in 2 hours.

4 c. c. of serum of rabbit No. 9 added to 50 c. c. of a 1 : 50 dilution of normal horse serum in physiological salt solution.

Allowed to stand 1 hour at 37° C., then 16 hours at room temperature.

Abundant precipitate, which was separated by centrifuging and washed four times with large amounts of normal salt solution.

2 c. c. of freshly drawn normal guinea pig serum added to this washed precipitate.

Allowed to stand 1 hour at 37° C.; then centrifuged and the serum separated from precipitate by centrifuging.

Normal G. P. No.	Injection.	Result.
349	1.8 c. c. normal guinea pig serum treated with washed precipitate as above, intravenously.	Marked dyspnoea at once; scratched typically in 20 minutes; quite sick for more than an hour.

Our results in the above few experiments confirm the authors above cited in demonstrating that acute anaphylactic shock may be produced in normal animals by the injection of suitable mixtures of antigen and anaphylactic antibody. The failure of the numerous similar experiments in which the serum of *sensitive* guinea pigs has been used, mixed with horse serum, is explained by the relatively small amount of allergin in the serum of sensitive guinea pigs as compared with "*immune*" guinea pigs.

Immediate anaphylactic shock in normal guinea pigs following the injection of mixtures of horse serum with specific antiserum seems capable of explanation only by the assumption of an anaphylactic antibody.

An explanation on this basis is that the anaphylactic antibody, acting upon its antigen (horse serum), produces in vitro a toxic substance (anaphylatoxin, apotoxin) which, by its action on vital cells, causes the symptoms of anaphylactic shock. The *precipitate*, when one is formed, may be considered as containing anaphylactic antibody and antigen in *concentrated* form. Normal guinea pig serum (containing complement) seems able to dissolve out the toxic substance from the precipitate.

Another possible explanation is that, by the action of the antibody (in presence of complement), the antigen is in some way rendered capable of *rapid* combination with the body cells. Whether the anaphylactic antibody acts as an intermediate link between antigen and body cells, or whether it serves to split up the antigen serum into *derivatives* capable of direct combination with the somatic cells, remains an open question.

THE EFFECT OF HEAT UPON ALLERGIN.

Lewis (25) has reported that allergin, or the substance in the serum of sensitive guinea pigs which will sensitize normal guinea pigs within 24 hours, is not destroyed by heating the serum to 60° C. for half an hour. We have some further work to report upon the effect of heat upon allergin. In addition to experiments to determine the effect of temperatures from 56° to 60° upon this body, we also report experiments designed to show whether the addition of the serum from normal guinea pigs to the heated sensitive guinea pig serum would increase its sensitizing property.

TABLE 28.—*Effect of heat on allergin.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
231	*Control:* 6 c. c. sensitive guinea pig serum, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	Marked symptoms.
232	6 c. c. sensitive guinea pig serum heated to 59-60° for 1¼ hours, intraperitoneally.	1	.....do..................	Slight symptoms.

TABLE 29.—*Effect of heat on allergin.—Added complement.*

Same lot of sensitive guinea pig serum as used in series 295–311.

G. P. No	First injection.	Interval in days.	Second injection.	Result.
292	*Control:* 3 c. c. sensitive guinea pig serum + 1 c. c normal guinea pig serum kept 1 hour at 37° C., intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	Marked symptoms.
293	3 c. c. sensitive guinea pig serum heated 1 hour 56–57° C. + 1 c. c. normal guinea pig serum, mixed, kept 1 hour at 37° C., intraperitoneally.	1	.....do................	No symptoms.
294	3 c. c. sensitive guinea pig serum heated to 56–57° C. for 1 hour, then kept at 37° C. for 1 hour, intraperitoneally.	1	.....do........	No symptoms.

TABLE 30.—*Effect of heat on allergin (56° to 58° C., half an hour).*

Serum obtained from 6 guinea pigs sensitized with toxin-antitoxin mixture.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
355	*Control:* 3 c. c. sensitive guinea pig serum, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	Severe symptoms.
356	*Control:* do...............................	1	.....do................	Very severe symptoms.
357	3 c. c. sensitive guinea pig serum heated to 56–58° C. one-half hour, intraperitoneally.	1	.....do................	Very severe symptoms.
358	.....do..................................	1	.....do................	Severe symptoms.
359	3 c. c. sensitive guinea pig serum heated 56–58° C. one-half hour+3 c. c. normal guinea pig serum, intraperitoneally.	1	.....do................	Slight symptoms.
360	.....do..................................	1	.....do................	Severe symptoms.
362	3 c. c. sensitive guinea pig serum heated 56–58° C.+2 c. c. normal guinea pig serum, intraperitoneally.	1	.....do................	Severe symptoms.
363	3 c. c. sensitive guinea pig serum heated 56–58° C.+1 c. c. normal guinea pig serum, intraperitoneally.	1	.....do................	Severe symptoms.
364	.....do..................................	1	.....do................	Severe symptoms.
365	3 c. c. sensitive guinea pig serum heated 56–58° C. one-half hour+0.1 c. c. normal guinea pig serum, intraperitoneally.	1	.....do................	Severe symptoms.
366	.....do..................................	1	.....do................	Severe symptoms.

NOTE. The normal guinea pig serum used was freshly obtained. After adding to heated sensitive serum, it was allowed to stand one hour.

TABLE 31.—*Effect of heat on allergin.*

The serum used was the mixed serum of 16 sensitive guinea pigs.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
396	3 c. c. sensitive guinea pig serum heated to 56–58° C. for 1 hour, intraperitoneally.	1	5 c. c. normal horse serum, intrapertioneally.	Marked symptoms.
397	.do..............................	1	...do..............	Marked symptoms.
398	3 c. c. sensitive guinea pig serum heated to 56–58° C. 1 hour + 1 c. c. normal guinea pig serum, intraperitoneally.	1	...do..............	Marked symptoms.
399	.....do..................................	1	.....do................	Marked symptoms.
400	3 c. c. sensitive guinea pig serum heated to 56–57° C. 1 hour + 2 c. c. normal guinea pig serum, intraperitoneally.	1	...do..............	Marked symptoms.
401	.do......	1	....do................	Marked symptoms.
403	3 c. c. sensitive guinea pig serum heated to 56–57° C. 1 hour + 3 c. c. normal guinea pig serum, intraperitoneally.	1	.....do................	Marked symptoms.
373	*Control.* 3 c. c. sensitive guinea pig serum, not heated, intraperitoneally.	1	....do..............	Very severe symptoms.
374	*Control:* do............................	1	....do..............	Very severe symptoms.

Notwithstanding a few irregular results, we are able to conclude quite definitely, in agreement with Lewis, that the anaphylactic antibody is not destroyed by a temperature of 56° to 58° C. for one hour. It would seem, however, that these temperatures in some cases impair its activity. We have not tried the effect of higher temperatures.

EFFECT OF LECITHIN IN PREVENTING ANAPHYLACTIC SHOCK.

Banzhaf and Steinhardt (6) reported that when 250 to 500 mg. or more of lecithin was given to serum-sensitized guinea pigs they were protected against a second injection of 5 c. c. of horse serum given 20 hours later. Whether by being "protected" they mean the prevention of the symptoms of anaphylaxis or the prevention of death is not clear. In order to confirm their statement the following series of experiments were made:

TABLE 32.—*Effect of lecithin injections into sensitive guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
407	0.01 c. c. normal horse serum............ 81 days later, 3 c. c. salt solution containing 0.25 gm. lecithin, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	Severe symptoms.[a]
408	.....do....................................	1	.....do................	Severe symptoms.[a]
409	*Control:* 0.01 c. c. normal horse serum, intraperitoneally.	81	.....do................	Dead in 20 minutes.
410	*Control:* do..............................	81	.....do................	Dead in 50 minutes.

[a] Nos. 407 and 408 immediately following the injection of lecithin showed restlessness, dyspnoea, fur ruffled, convulsive movements, sick for several hours; then recovered completely. Symptoms on the injection of horse serum were severe, but not typical; chiefly dyspnoea.

TABLE 33.

The lecithin used in this experiment was the same as that used for G. P. Nos. 393, 394, 407, and 408 after having been purified.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
411	0.01 c. c. normal horse serum, subcutaneously. 82 days later, 0.25 gm. purified lecithin in 4 c. c. salt solution, subcutaneously.	1	5 c. c. normal horse serum, intraperitoneally.	Marked symptoms.
412	0.01 c. c. normal horse serum, subcutaneously. 82 days later, 0.25 gm. purified lecithin in 4 c. c. salt solution, intraperitoneally.	1	.....do.................	No symptoms.
413	0.01 c. c. normal horse serum, subcutaneously. 95 days later, 0.25 gm. lecithin in 4 c. c. salt solution, subcutaneously.	1	.....do.................	Dead in 36 minutes.
414	.....do....................................	1	.....do.................	Very severe symptoms.

NOTE.—G. P. Nos. 412, 413, and 414 showed slight symptoms of discomfort on injection of lecithin. Sick several hours; all right in 24 hours.
G. P. No. 411 showed no symptoms on injection of the lecithin.

TABLE 34.—*Effect of lecithin in preventing passive anaphylaxis.*

The sensitive guinea-pig serum used in this experiment was the same as that given to G. P. Nos. 373 to 393 (see Table No. 11), which serve as controls for the activity of this serum.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
394	3.5 c. c. sensitive guinea-pig serum+0.25 gm. lecithin emulsified together and left 1 hour at room temperature, intraperitoneally.	1	5 c. c. normal horse serum, intraperitoneally.	No symptoms.
395	do.[a]	1	do.	No symptoms.
415	4 c. c. sensitive guinea-pig serum+0.1 gm. lecithin emulsified together and left 1 hour at room temperature, intraperitoneally.	1	do	Mild symptoms.
416	do	1	do.	Mild symptoms.
417	*Control:* 4 c. c. sensitive guinea-pig serum, intraperitoneally.	1	do.	Slight symptoms.

[a] Two guinea pigs, sensitized 95 days previously by an injection of 0.01 c. c. normal horse serum, were bled, blood defibrinated, centrifuged, and serum used.

NOTE.—Nos. 415 and 416 showed symptoms immediately on injection of lecithin. Sick several hours, but recovered in 24 hours.

The results of our experiments fail to confirm Banzhaf and Steinhardt, since five out of six sensitive guinea pigs had severe symptoms and one died from the injection of 5 c. c. of horse serum 24 hours after receiving 0.25 gm. of lecithin.

Our results, as regards the neutralization of horse serum allergin by lecithin, are such that no definite conclusions can be drawn from them. Two guinea pigs each received 3.5 c. c. of sensitive guinea-pig serum +0.25 gm. of lecithin and failed to show symptoms of anaphylaxis 24 hours later when injected with 5 c. c. of horse serum. Two other guinea pigs receiving 4 c. c. of sensitive guinea-pig serum +0.1 gm. lecithin developed passive anaphylaxis, showing more marked symptoms than the control which received no lecithin.

Both sensitive and normal guinea pigs constantly showed immediate symptoms somewhat resembling anaphylactic shock when 0.1 to 0.25 gm. lecithin was injected intraperitoneally, either with salt solution or with guinea-pig serum. These symptoms, however, may readily be accounted for by local irritation. After subcutaneous injections of lecithin the guinea pigs showed only slight indefinite signs of discomfort.

The lecithin used was egg lecithin, which had been kept for some time, showed acid reaction, and probably contained some impurities. This was purified, and the purified lecithin was used in the experiments on G. P. Nos. 411 and 416.

PASSIVE TRANSFER OF ANAPHYLAXIS FROM OTHER ANIMALS TO GUINEA PIGS.

Otto was the first to show that anaphylaxis could be transferred passively to guinea pigs by the serum of an animal of another species (rabbit) that had received an injection of serum at least 14 days previously.

Anderson and Rosenau (41) have reported negative results in attempts to passively sensitize guinea pigs with the serum of human beings, monkeys, and cats which had previously received an injection of horse serum.

Novotny and Schick (33) report the successful transferrence of passive anaphylaxis to guinea pigs with the serum of two children who had received injections of horse serum 17 and 23 days, respectively, previous to being bled, these being, however, their only positive results in a considerable number of experiments. They report negative results in the attempt to transfer anaphylaxis passively from dog to rabbit, and from guinea pig to rabbit.

We now wish to report some work upon the passive transfer of anaphylaxis from various animals to guinea pigs.

TABLE 35.—*Passive transfer of horse serum anaphylaxis from man to guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
321	Intraperitoneally, 3 c. c. serum of man who had received two injections of horse serum as follows: In 1900, 10 c. c. Yersin serum. In October, 1909, 4 c. c. tetanus antitoxin, followed by severe rash on seventh day. Bled April 10, 1910.	1	5 c. c. normal horse serum, intraperitoneally.	Slight symptoms.[a]
322	.....do	1	.....do	Mild symptoms.[a]

[a] Both these guinea pigs showed symptoms after 45 minutes, increasing in severity after 1 hour.

TABLE 36.—*Passive transfer of anaphylaxis from cat and donkey to guinea pig.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
367	Intraperitoneally, 3 c. c. serum of cat which had received 5 c. c. horse serum 33 days previously.	1	5 c. c. normal horse serum, intraperitoneally.	Symptoms.
368	.....do	1	.....do	Marked symptoms.
371	Intraperitoneally, 3 c. c. serum of donkey which had, 8 years previously, been repeatedly injected with hog serum.	1	5 c. c. hog serum, intraperitoneally.	No symptoms.
372	.....do	1	.....do	No symptoms.

The successful transference of anaphylaxis from man to the guinea pig indicates a close relation between acquired serum hypersusceptibility in man and in the guinea pig, notwithstanding the markedly different reactions obtained in man and in the guinea pig. The same may be said as regards the transfer of anaphylaxis from the cat to the guinea pig, the cat being relatively insusceptible to anaphylactic reaction.

The failure to passively sensitize guinea pigs with the donkey serum used may easily be due to the long lapse of time (8 years) since the serum treatment of this donkey.

THEORETICAL DISCUSSION OF ANAPHYLAXIS.

The very considerable additions recently made to our knowledge of the phenomenon of anaphylaxis, together with the enormous number of observations already accumulated, present a mass of facts which, considered as disconnected facts, are all but impossible to keep in mind. And yet any attempt to correlate these observations into a theory of the nature and mechanism of anaphylaxis very soon reveals wide gaps in the chain of facts necessary to satisfactorily establish any such theory. Notwithstanding, however, the attempt to arrange the facts already known into a connected theory seems not only justifiable, but even necessary, in order (1) to render the facts easier of retention by means of their relation and (2) to indicate more clearly the gaps as subjects for future experiments.

With these objects in view, we present a brief review of some theories of anaphylaxis in their relation to the observed facts, together with the partial conclusions at which we have arrived, with no desire to dogmatize nor to insist upon the details of this complex mechanism, realizing that in the present state of our knowledge anything more than a general outline of the principles of the reaction is impossible.

SPECIFIC ANAPHYLACTIC ANTIBODIES.

The experiments of Schultz (44), already referred to as demonstrating an increased stimulation of the unstriped muscle of sensitive guinea pigs by horse serum, seem to us to signify clearly one of two conditions—either increased "affinity" of the tissue of a sensitive guinea pig for the sensitizing antigen or an increased combining apparatus, more numerous receptors, capable of combining with horse serum. As it is difficult to conceive of increased specific affinity independent of molecular change, it seems more reasonable to ascribe the increased action of horse serum upon sensitive smooth muscle to a specific change in the latter, corresponding to Ehrlich's conception of an increase in its combining receptors for horse serum.

This is equivalent to assuming the existence of "antibodies" in the broad sense of the word that is, a combining apparatus the result of reaction to the antigen and specific for this antigen. In this particular instance the specificity of the reaction in question requires further study.

The presence of antibodies free in the serum of sensitized guinea pigs (and other animals studied) is indicated chiefly by the following facts:

(1) The passive transferrence of anaphylaxis. The recent demonstrations that the passive transfer of anaphylaxis is quantitative and that the incubation period is shorter than previously supposed increase the demonstrable analogy between passive transfer of anaphylaxis and the passive transfer of other antibodies.

(2) The prevention of passive sensitization by the addition of antigen to sensitive serum. This would seem to indicate very clearly the saturation of antibody.

(3) The production of immediate anaphylactic shock in normal guinea pigs by the injection of mixtures of "immune" serum and antigen. The fact that this effect has been demonstrated only with the serum of "immunized," rather than sensitized, guinea pigs is quite in harmony with the abundant demonstrations that the specific sensitizing substance (antibody) is more abundant in the blood of immune than of sensitive guinea pigs. A specific antibody in the serum of sensitive guinea pigs, acting in the same direction, had already been indicated by the work of Rosenau and Anderson (41), who showed that the toxicity of horse serum for sensitive guinea pigs is increased by the addition *in vitro* of sensitive guinea pig serum.

RELATION OF ANAPHYLACTIC ANTIBODY TO PRECIPITIN.

The relation of the anaphylactic antibody to precipitin has been emphasized within the last year by Friedberger (15), who has formulated a theory based upon this relationship.

Another theory, correlating the anaphylactic antibody with precipitin, but in a different manner, had previously been advanced by Nicolle (30, 31, 32).

The primary grounds of both these theories are:

(1) The identity of the antigen concerned in causing hypersusceptibility to a proteid and the formation of precipitins for this proteid. The recent very excellent work of Doerr and Russ (11, 12) and of Doerr and Moldovan (13, 14) has very strongly supported this assumption of the identity of the antigen concerned in sensitization and in precipitin formation.

(2) The relation between free precipitin and acquired immunity to anaphylactic shock. It has long been recognized that the same treatment (repeated, relatively large injections of proteid) which renders an animal (especially the guinea pig) immune to anaphylactic shock also favors the formation of free precipitins for the antigen in the animal's blood.

Friedberger's theory of anaphylaxis is briefly as follows:

When a foreign proteid is injected into the body of an animal the organism reacts by the formation of specific combining receptors. These receptors are at first sessile (or intracellular), and under the moderate stimulation of a single small injection of the antigen remain for the most part sessile, especially so in the guinea pig. Greater stimulation by repeated injections of antigen causes a large excess of the same specific receptors to be thrown off free into the blood stream. These receptors, whether sessile or free, are identical with the precipitin which is demonstrable in the blood of suitably treated animals.

According to this theory a normal guinea pig is insusceptible to horse serum, for instance, because it possesses few receptors with which this serum may combine. A sensitized guinea pig is hypersusceptible to horse serum because it possesses numerous *sessile* (intracellular) receptors, enabling the proteid to combine rapidly with the cells. An "immune" guinea pig is insusceptible to the action of horse serum because it possesses enough *free* receptors to combine with the antigen and so deflect it from the sessile receptors. Friedberger assumes that in a sensitive guinea pig it is the combination of *sessile precipitin* with its antigen (precipitable substance) which causes anaphylactic shock; that in the blood of a sensitive guinea pig free precipitins are present in sufficient quantity to passively transfer anaphylaxis, but not in sufficient quantity to cause a visible precipitate *in vitro*. He explains the lapse of time necessary after injection of "sensitive" serum into a fresh animal before the latter becomes sensitive as the time necessary for the injected free precipitin to become fixed to somatic cells or sessile. The combination between precipitin and somatic cells (fixation) is believed to be a much slower reaction than the combination of precipitin with the precipitable substance of the antigen.

This theory has since been supported by further observations:

(1) The observations of Doerr and Russ (12), and of Doerr and Moldovan (13), that the passive sensitizing strength (= amount of sensitizing antibody) in a specific antiproteid serum is proportional to its precipitating strength.

(2) The observation (also first made by Doerr and Russ (12)) that the *precipitate* formed by the action of a specific precipitating serum upon its antigen, when redissolved in weak soda solution and injected into fresh guinea pigs, causes in them immediate anaphylactic shock, indicating that the precipitate (precipitin + precipitable substance) contains a combination of antigen plus anaphylactic antibody which, by combination (fixation) with somatic cells, causes anaphylactic shock. The slow onset and course of symptoms following the intravenous injection of this antigen-antibody combination was explained by Doerr and Russ as due to the comparatively slow fixation of antibody to somatic cells.

(3) The observation, made by Friedberger (17), that sensitive guinea pigs can sometimes be protected from anaphylactic shock by injecting a large amount of precipitating rabbit serum shortly before injecting the antigen.

Nicolle (30, 31, 32) assumes that when a foreign proteid is injected into the body of an animal the organism reacts by the formation of *two* specific antibodies. One of these is an *albumino-lysin* whose action is to split up the antigen as a necessary step in its destruction and assimilation. This splitting up, however, liberates from the proteid certain "endotoxins" which, if liberated in excessive amount or with excessive rapidity, have an intoxicating action upon the body cells. The albumino-lysins are amboceptors, requiring the aid of "complement" for their action. The other antibody is an *albumino-coagulin* (precipitin) whose action *in corpore* is to condense the antigen (*in vitro* to precipitate it) rendering it less readily acted upon by the albumino-lysin. This antibody, precipitin, does not require the presence of complement to activate it.

Although both these antibodies are always formed in the reaction to a proteid antigen, their relative proportions may vary widely, depending upon (1) the nature of the antigen, (2) the amount and manner of administering it, and (3) the specific or individual characteristics of the animal. An animal that reacts by the formation of a preponderance of albumino-lysin is thereby rendered hypersusceptible to the antigen, being now capable of splitting up the antigen more rapidly than normal, thus liberating the "endotoxins" more rapidly. If, however, the albumino-coagulin (precipitin) has been formed more abundantly than the albumino-lysin, the animal is "immune," because antigen now introduced into the body is at once rendered "insoluble" by the action of the "precipitin," albumino-lysis is retarded, and anaphylactic shock thus prevented.

In general, the reaction to repeated large amounts of antigen tends toward the formation of an excess of the protective albumino-coagulin, while the slight reaction to a single antigen injection tends toward preponderance of the albumino-lytic antibody. According to Nicolle's theory, the anaphylactic antibody is an albumino-lysin, whose action upon the antigen is directly opposed to the action of precipitin.

The observations with which Nicolle supports his theory are:

(1) Observations on the lytic and "coagulating" antibodies produced by antigens other than nontoxic proteids, for instance by soluble toxins, blood corpuscles, bacteria, etc., observations which can not be discussed in this space.

(2) The demonstration in the serum of serum-sensitized guinea pigs, where no precipitin can be demonstrated, of an antibody (lysin) capable of deviating complement in the presence of its antigen. This observation lacks satisfactory confirmation.

Further observations made since Nicolle's publication, which tend to confirm this theory, are:

(1) The observation that *normal guinea pig serum* treated with the *washed precipitate* obtained by adding an antigen proteid to specific antiserum, produces the symptoms of anaphylactic shock when injected into a *normal* guinea pig. This seems capable of explanation by supposing that in the presence of complement the albumino-lysins (which may be carried down in the precipitate) have split up the

antigen into toxic constituents. It is difficult to explain this reaction on the assumption that the (intracellular) combination of precipitin with precipitable substance is the essential reaction in producing anaphylactic shock, since this reaction has already taken place *in vitro*.

Comparing the above two theories, it is seen that they agree in assuming:

(1) That a specific antibody, the result of reaction to the sensitizing antigen, is the essential factor in the condition of anaphylaxis.

(2) That anaphylactic shock is due to the combination of the antigen (or some derivative of the antigen) with somatic cells, and that the specific antibody is necessary to effect this combination.

(3) That the same antigen causes the formation of anaphylactic antibody and precipitin.

(4) That free precipitin in the blood is the protective agency in immunity.

They differ radically, however, in their conception of the relation between the anaphylactic antibody and precipitin, Friedberger assuming that the two are *identical*, while Nicolle assumes that they are essentially opposite. Friedberger assumes as the essential difference between a hypersusceptible and an immune animal the *location* of the *same antibody* (precipitin), this being sessile in the case of hypersusceptibility, free in the case of immunity; while Nicolle believes that the distinguishing factor between hypersusceptibility and immunity is the *nature* of the preponderating antibody, albumino-lysin (anaphylactic antibody) or albumino-coagulin (precipitin) respectively.

The points wherein these theories agree may be considered as justified by experimental evidence. The evidence that precipitin, free in the blood, is the protective agency in "immune" animals seems to be as yet insufficient to establish the fact, although sufficiently suggestive to make the hypothesis reasonable. The disagreements of the theories may be taken as indicating gaps in the chain of experimental evidence. It has not been satisfactorily demonstrated that the antibody of the cellular tissues and serum of sensitive guinea pigs is identical with precipitin, as Friedberger claims; nor, on the other hand, has there been any satisfactory demonstration of the *two* (separable) antibodies which Nicolle assumes.

There is still, admittedly, ample range for considerable differences of opinion, dependent on the personal equation in correlating and interpreting the facts on which these and other theories of anaphylaxis are based. The present opinions concerning the mechanism of anaphylaxis seem, however, to be at least no more widely divergent than the opinions concerning other immunity reactions; and seem, moreover, to be converging in essential principles, even when diverging widely in detail.

OUR VIEWS OF THE MECHANISM OF ANAPHYLAXIS.

In conclusion, we present a synopsis of our present views of the mechanism of anaphylaxis, acknowledging our indebtedness to all available literature on the subject.

SENSITIZATION.

When a foreign proteid is taken into the body in the normal way, through the alimentary canal, it is believed that it is split up by the action of the digestive juices and specialized cells into simpler compounds, and that these *protein derivatives* are supplied to the general body tissues for assimilation. It may be assumed, then, that the body cells are adapted for combining with such protein derivatives, possess, in other words, suitable receptor apparatus for the assimilation of such forms of protein derivatives as are normally supplied to them.[a]

The tissues outside of the alimentary canal are presumably, however, not supplied with a very abundant receptor apparatus for *whole* foreign proteids. When, therefore, a whole foreign proteid is introduced into the general circulation (by subcutaneous, intraperitoneal, or intravascular injection) it is probably assimilated very slowly, owing to scarcity of receptors capable of combining with it. According to the principles of Ehrlich's theory the reaction of the organism in such a case would be in the direction of forming *more* of the receptors needed for assimilating and disposing of this foreign proteid.

It seems at least reasonably probable that the first step in this process of assimilation is a splitting up of the foreign proteid molecule, and that this preliminary splitting up is the process for which the *normal* tissue cells (and perhaps the blood and lymph) are poorly adapted; that the antibody formed is therefore proteolytic, designed to split up the foreign proteid into forms capable of combining with the food receptors of the tissue cells.

THE ANAPHYLACTIC ANTIBODY.

Experimental evidence indicates that specific receptors (antibody) exist both in the cellular tissues and in the serum of actively sensitized animals. As to whether the sessile (intracellular) antibody is the same as the free antibody, only a surmise can be made. The action of this antibody apparently needs to be aided by the nonspecific "complement" of the blood, as indicated by the decrease in complement during anaphylactic shock and by the toxic action of precipitate treated with normal guinea pig serum (complement).

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[a] Wells (49) has recently reported that guinea pigs sensitized with urine from four healthy persons with experimental albuminuria produced by eating raw eggs, reacted typically and severely to subsequent injections of human ascitic fluid, but not to egg-white; showing that even under such abnormal conditions foreign proteid undergoes change in the process of elimination, losing its original specific characteristics and acquiring characteristics specific for the assimilating organism. As Wells suggests, further studies, along this line, of both blood and urine promise valuable information as to the state in which proteids pass from the alimentary canal into the blood, a point on which definite information is still lacking.

The experiments of Schultz (44), on the other hand, indicate that horse serum may act upon smooth muscle fiber without the aid of serum-complement.

The structure of the antibody concerned is, of course, unknown, as is also the nature of the chemical changes produced by it. Its action, however, is quantitative. (Cf. Saturation of antibody *in vitro.*) It may be reasonably conjectured that its action is proteolytic for the following reasons:

(1) From analogy: Proteid assimilation from the digestive tract is normally preceded by proteolysis. "Organized" proteids undergo "lysis" (cytolysis-bacteriolysis) when injected into the circulation.

(2) Certain *derivatives* of harmless proteids injected directly into the circulation cause symptoms similar to anaphylactic shock. (Biedl and Kraus (8), peptone in dogs; Vaughan and Wheeler (48), split proteids in guinea pigs.) Especially significant in this connection, if confirmed, is the observation of Biedl and Kraus that sensitive dogs reacting to peptone become anti-anaphylactic to the specific sensitizing antigen.

(3) Recent researches of Doerr and Moldovan (14) have shown that when ox serum is precipitated by specific rabbit serum in suitable proportions, neither the precipitate nor the supernatant fluid will actively sensitize guinea pigs in 10 days, although both precipitate and fluid will sensitize to rabbit serum, and the amount of ox serum originally present in the mixture was greatly in excess of the amount necessary to sensitize a guinea pig.

If antigen is in *excess*, the fluid, but not the precipitate, will sensitize to ox serum. This would seem to indicate that the amount of antigen (ox serum) used had been altered in some way by the action of the specific antiserum so as to loose its specific and antigenic properties. Taking this fact in connection with the observed toxicity of guinea-pig serum treated with precipitate and of precipitate alone, the inference is reasonably clear that the antigen was split up by the action of the rabbit serum antibody.

ANAPHYLACTIC SHOCK.

Anaphylactic shock is, we believe, a disturbance of metabolic equilibrium, due to temporary deflection of the normal metabolic activity of the tissue cells, rather than the result of any specific toxic action, because—

(1) The symptoms produced by the reaction to various antigens are strikingly similar.

(2) The symptoms are also strikingly similar to the symptoms of asphyxiation, which is essentially a general disturbance of metabolic equilibrium.

(3) The severity of the shock in animals of the same degree of hypersusceptibility is proportional to the rapidity with which the antigen is thrown into the circulation.

(4) Recovery from anaphylactic shock is rapid and complete, leaving nothing to indicate the action of a "toxin."

(5) Antianaphylaxis may result from the slow absorption of antigen without anaphylactic shock.

(6) The reaction of sensitive smooth muscle to horse serum *in vitro* is simply an *exaggeration* of the reaction of normal muscle, showing, between this particular tissue and the specific antigen, a reaction of the "explosive" nature which we assume to be the distinguishing characteristic of anaphylactic shock.

The production of anaphylactic shock by normal guinea-pig serum treated with precipitate does not necessarily indicate the action of a toxin (anaphylatoxin). It may equally well be explained by the splitting up *in vitro* of the whole proteid into products which combine immediately (explosively) with normal cells.

It is not necessary to assume any *specific* toxic action either upon nerve centers or contractile tissues. Assuming that the functions of *all* cells are deflected during anaphylactic shock, we would still expect *objective symptoms* only from the disturbance of motor (and sensory) centers and contractile tissues. We should expect death to be due to the disturbance of the most vital functions—respiration and circulation. Anaphylactic shock is not identical with the *toxic* action exhibited by toxic sera.

ANTIANAPHYLAXIS.

Antianaphylaxis is the state which, in an anaphylactic animal, immediately succeeds anaphylactic shock. Provided the dose of antigen producing anaphylactic shock has been sufficient, antianaphylaxis is believed to be a state of absolute immunity to anaphylactic shock from a further injection of the same antigen. Antianaphylaxis is specific, and its development is regarded as one of the essential characteristics of anaphylaxis in the guinea pig. The condition of antianaphylaxis may, however, be induced without actual anaphylactic shock by the injection of antigen in small repeated doses, or in such ways that it may be *slowly* absorbed. The end-result in this case being the same as the end-result after anaphylactic shock, we are led to infer that essentially the same reaction has taken place in both cases, in one case so rapidly as to cause symptoms by disturbance of metabolic equilibrium and in the other case so gradually as to cause no disturbance of this equilibrium sufficient to give rise to objective symptoms.

Partial antianaphylaxis results from a dose of antigen too small to cause complete antianaphylaxis. A partially antianaphylactic animal reacts toward another injection of antigen precisely as does a sensitive animal, only less severely.

Antianaphylaxis may be explained as due to the saturation of anaphylactic antibody with antigen, a view which is supported by our failure to passively sensitize fresh guinea pigs with the serum of guinea pigs recently recovered from anaphylactic shock (antianaphylactic).

IMMUNITY.

The absolute insusceptibility characteristic of antianaphylaxis is a temporary condition, passing gradually into a condition of *relative* insusceptibility, which we distinguish as "immunity" proper, which immunity, under certain conditions, gradually disappears, leaving the animal finally hypersusceptible.

Immunity, as here defined, is distinguished from antianaphylaxis by two demonstrable factors:

(1) The presence of free anaphylactic antibody in the blood in larger amount even than in the blood of a sensitive animal, as has often been shown by passive sensitization with the blood of immune animals.

(2) A relative insusceptibility to anaphylactic shock instead of the absolute insusceptibility of antianaphylaxis. It has been shown that an immune guinea pig will react to the specific antigen with anaphylactic symptoms under certain conditions. The severity of this reaction increases, up to certain limits, with the *length* of time that has elapsed since receiving the immunizing antigen. It is proportional also to the *amount* of antigen in the immunizing dose or doses.

An animal may then be insusceptible to anaphylactic shock in three cases:

(*a*) A normal animal, because of *scarcity* of combining receptors.

(*b*) An antianaphylactic animal, because of saturation of combining receptors.

(*c*) An immune animal, the protective mechanism in this case being still a matter of conjecture, but at least not capable of explanation by either of the above two hypotheses.[a]

Taking the case of a guinea pig which has recovered from a second injection of horse serum: After a sufficient length of time the blood of this animal contains abundant free anaphylactic antibody, more than the blood of a highly sensitive guinea pig, yet the animal is very slightly susceptible to anaphylactic shock. The explanations offered are:

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[a] It is to be remembered that "immunity" is a transition stage between antianaphylaxis and reacquired hypersusceptibility, being separated from neither of these by any sharp line.

(1) Friedberger's explanation that the free antibody in the blood is sufficient to combine with antigen which may now be injected, thus protecting the sessile antibody and averting anaphylactic shock. Consideration of the quantitative relations shown to exist between the antigen and antibody (see Tables 4–8) taken in connection with the quantitative estimation of the free antibody in the blood of immune animals make it seem highly improbable that enough free antibody exists to combine with more than a small fraction of a cubic centimeter of horse serum, while the animal may actually be refractory toward a much larger dose than this. Provided the action of complement can be proven to be necessary to the anaphylactic reaction, the assumption of protection by excess of anaphylactic antibody deflecting the complement will have more probability.

(2) Nicolle's explanation, that albumino-coagulins (precipitins) have developed in sufficient amount to retard the action of the albumino-lysin which produces anaphylactic shock. The gap in this argument is the failure to satisfactorily demonstrate the existence of two such opposing antibodies by separating one from the other.

(3) Other theoretical possibilities, such as a change in the receptor apparatus of the *cells*, have not been excluded by experimental evidence.

It would be of great value, in this connection, to ascertain whether the muscle of an immune guinea pig will react to horse serum in the same way and degree as the muscle of a sensitive guinea pig.

PASSIVE ANAPHYLAXIS.

The passive transfer of anaphylaxis has offered at the same time some of the best arguments for and against the theory of an antibody being the essential factor in the condition of anaphylaxis.

The undisputed facts with regard to passive anaphylaxis are:

(1) Normal guinea pigs may be rendered hypersusceptible to a specific proteid by transference of serum from another animal (homologous or heterologous) which has been sensitized or immunized to this proteid. The substance which passively sensitizes is more abundant in the blood of "immune" animals than in the blood of animals sensitized by a single injection of serum.

The blood corpuscles and somatic tissues of a sensitive guinea pig do not passively sensitize a normal guinea pig.

(2) Given a constant interval of 24 to 48 hours the *degree* of passively acquired hypersusceptibility is proportional to the amount and concentration of sensitive serum (anaphylactic antibody) received.

(3) The simultaneous injection of sufficient antigen, along with sensitizing serum (anaphylactic antibody) prevents passive hypersusceptibility from developing within 48 hours.

(4) A certain interval of time must elapse after the injection of a sensitive serum (even homologous serum) into a normal guinea pig before the latter develops its *maximum* degree of hypersusceptibility. Just what this interval of time is remains insufficiently determined.

Recent researches demonstrating immediate anaphylactic shock in normal animals upon the intravenous injection of sufficiently concentrated mixtures of antigen and antianaphylactic antibody indicate that if any change in the somatic tissues is necessary in the development of passive anaphylaxis (as seems highly probable) this change *may* take place very rapidly. But while a certain degree of passive hypersusceptibility may be acquired almost immediately after injection of anaphylactic antibody into the circulation it has not been claimed by anyone that the *maximum* of passive hypersusceptibility is reached so soon.

The least intervals of time cited as necessary for the development of the maximum degree of passive hypersusceptibility in a normal guinea pig, after the injection of sensitive serum, are: 4 hours after intravenous injection; 12 to 14 hours after intraperitoneal injection; 24 to 48 hours after subcutaneous injection.

Gay and Southard, on the other hand, in their work on passive anaphylaxis pointed out that the maximum degree of passive hypersusceptibility was not reached until after a longer interval (5 to 15 days) after injection of sensitive serum. This observation was accepted and confirmed by Otto and Lewis. Experiments given in this bulletin (p. 34) also support this claim. Observations conflicting with these have, however, been reported. Otto found that guinea pigs which had received serum from a rabbit treated with horse serum reacted to horse serum in 2 days and in 5 days. The single guinea pig tested after 15 days failed to react to horse serum. Lewis found that normal guinea pigs which had received the serum of congenitally sensitive guinea pigs reacted to the specific antigen after 17 to 36 hours, but not after 19 days.

Rosenau and Anderson, in a series of experiments on passive anaphylaxis, found that guinea pigs tested 48 hours after receiving sensitive guinea-pig serum reacted more constantly than those tested 26 days after receiving the sensitive serum.

In view of these conflicting observations it is evident that the influence of time, beyond the time necessary for absorption, upon the development of passive anaphylaxis, has not yet been determined with sufficient exactness to warrant dogmatic conclusions on this point. The more recent researches, however, have tended to confirm the opinion that the passive transfer of hypersusceptibility is a transfer of the specific antibody.

RELATION OF ANAPHYLAXIS IN GUINEA PIGS TO REACTIONS OF OTHER ANIMALS TOWARD FOREIGN PROTEIDS.

The phenomenon of anaphylaxis in the guinea pig presents apparently great differences from the phenomenon as exhibited in other animals. The progress of investigations upon other animals is, however, constantly tending to show that anaphylaxis in the guinea pig differs from that of other animals mainly in a *quantitative* sense; that in the other animals studied the *nature* of the reaction is essentially the same, even though the *manifestations* are widely different. Especially convincing in this connection is the passive sensitization of the guinea pig by the serum of various animals (rabbit, cat, dog, and man), which themselves react to the antigen very differently from the guinea pig.

SUMMARY AND CONCLUSIONS.

The word "allergin" is used to designate an antibody characteristic of anaphylaxis, specific for its antigen, and which we regard as the essential agent in the passive transference of anaphylaxis.

1. It was found that 3 c. c. of serum from a guinea pig sensitized by a single small injection of horse serum contained sufficient allergin to constantly sensitize normal guinea pigs to an injection of horse serum 24 hours later.

2. The least amount of horse serum required to neutralize the allergin in 3 c. c. of sensitive guinea pig serum was 0.0005 c. c. In some cases 0.001 c. c. to 0.005 c. c. was required. In all instances 0.01 c. c. was sufficient.

3. The same effect was obtained whether the horse serum and the sensitive guinea-pig serum were mixed and allowed to stand 1 hour *in vitro* before injection, injected as soon as mixed or injected separately at the same time.

4. Horse serum has no effect in preventing passive sensitization to egg-white, whereas 0.000001 gram of pure egg-white, injected with guinea-pig serum containing egg-white allergin, is sufficient to neutralize the latter.

5. When guinea pigs were given 0.01 c. c. of horse serum followed at various intervals by the injection of 3 c. c. of sensitive guinea-pig serum, passive sensitization was prevented when the interval was not greater than 12 hours.

6. When the interval was longer (15 to 18 hours) passive hypersusceptibility was modified, but not constantly prevented. It would seem from this that the amount of serum used (0.01 c. c.), within 12 to 48 hours after the injection into the body of a normal guinea pig, had undergone such changes (probably at least the preliminary steps toward assimilation) as to be appreciably diminished.

7. When normal guinea pigs were given 3 c. c. of sensitive guinea-pig serum and, at various intervals up to 24 hours, 0.01 c. c. of normal horse serum, passive anaphylaxis was prevented in those animals which received the horse serum within 6 hours after receiving the sensitive guinea-pig serum. In the remainder of the series passive anaphylaxis was either prevented or modified. This would seem to indicate that within 6 hours the allergin had become "bound" in some way, so that it could no longer be neutralized by the injection of horse serum in the amount used by us.

8. It was found that when normal guinea pigs were given 3 c. c. of serum from guinea pigs sensitive to horse serum, along with varying amounts of normal serum of different animals, 0.001 c. c. of horse serum was sufficient to prevent passive sensitization; it required 0.1 c. c. of donkey serum; 1 c. c. of goat serum, sheep serum, and hog serum caused doubtful diminution of hypersusceptibility; 0.1 c. c. of each of these sera caused no diminution. Of the sera from man, rabbit, dog, cat, chicken, ox, and of milk, 1 c. c. caused no diminution in the hypersusceptibility as compared to the controls. The effect of horse serum, then, as compared to donkey serum, is as 100 : 1; as compared to other sera and to milk, at least 1,000 : 1.

9. Mixtures of 3 c. c. of sensitive guinea-pig serum with varying amounts of the specific antigen sensitized normal guinea pigs when tested 19 days later.

10. Attempts to absorb the anaphylactic antibody from sensitive guinea-pig serum by 4-hour contact with the various organs of normal or sensitive guinea pigs were not successful. The amount of allergin was apparently not diminished by this procedure.

11. We were unable to passively sensitize guinea pigs by injecting an emulsion of the brain or kidney of a sensitive guinea pig or by injecting the washed corpuscles of a sensitive guinea pig.

12. Guinea pigs during the latter stage of anaphylactic shock and shortly thereafter do not have in their serum sufficient free allergin to passively sensitize normal guinea pigs. At long intervals after recovery from anaphylactic shock (17 to 450 days), recovered guinea pigs do contain free allergin in their serum, even when themselves very slightly susceptible to anaphylactic shock. These experiments indicate the saturation of allergin taking place during anaphylactic shock.

13. The quantitative estimation of allergin in the serum of sensitive guinea pigs, recovered guinea pigs, and rabbits treated by repeated injections of serum showed that 3 c. c. of serum of guinea pigs, sensitized more than 30 days previously with a single injection of horse serum, is approximately the minimum amount that will constantly passively sensitize normal 250-gram guinea pigs to an intraperitoneal injection of horse serum 24 hours later. The serum of

immune guinea pigs contains very considerably more allergin—at least three times as much—in our experiments. The serum of a rabbit treated with frequent large injections of horse serum contained more free allergin than the serum of highly sensitive guinea pigs.

14. It was found that the degree of hypersusceptibility conferred by equal amounts of sensitive serum was greater after 15 days than after 1 day. Even after 15 days, however, our guinea pigs were not sensitized by less than 1 c. c. of sensitive serum.

15. It was found that immediate anaphylactic symptoms could be produced in normal animals by the injection of suitable mixtures of antigen and anaphylactic antibody.

16. Temperatures of 56° to 58° C. for one hour do not destroy the anaphylactic antibody in sensitive guinea-pig serum; but it would seem from our experiments that these temperatures in some cases impair its activity.

17. Allergin was successfully transferred from man and the cat to the guinea pig.

18. The administration of 0.25 gram of egg lecithin to sensitive guinea pigs 24 hours before the intoxicating injection did not appreciably modify the development of anaphylactic shock.

19. We present the following synopsis of our views of anaphylaxis in the guinea pig:

Hypersusceptibility to a foreign proteid consists in an increase in the normal power of assimilating this proteid, especially an increase in the rapidity of the reaction. This is due to the formation of a specific antibody (or antibodies) demonstrable in somatic tissues (smooth muscle) and in the serum of sensitive guinea pigs. The action of this antibody upon its antigen is quantitative and is probably primarily proteolytic.

Anaphylactic shock is due to disturbance of metabolic activities of vital cells rather than to the specific (permanent) intoxication of cells.

Antianaphylaxis is a state of insusceptibility due to saturation of the specific combining receptors.

"Immunity" is a state of relative insusceptibility, characterized by increase in the amount of free anaphylactic antibody. Whether the insusceptibility of an immune guinea pig is due to this excess of free anaphylactic antibody, to the formation of another free antibody, or to specific changes in the somatic cells is an open question.

The passive transfer of anaphylaxis, as also its hereditary transmission, is a transfer of the anaphylactic antibody.

Anaphylaxis is a step toward immunity, which is conceived as an increased capacity for safely and rapidly eliminating the specific antigen proteid. It is remarkable among immunity reactions chiefly in the fact that (in the guinea pig especially) this stage of hypersusceptibility is strikingly constant, well defined, and of long duration.

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LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis.* By M. J. Rosenau.

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*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, oy S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi.* By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

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*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth decennial revision. By Reid Hunt and Murray Galt Motter.

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No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species *Paragordius varius*), with summary of all cases reported to date. By Ch. Wardell Stiles.

*No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

No. 39.—The antiseptic and germicidal properties of solutions of formaldehyde and their action upon toxines. By John F. Anderson.

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No. 45.—Further studies upon anaphylaxis. By M. J. Rosenau and John F. Anderson.

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TREASURY DEPARTMENT

Public Health and Marine-Hospital Service of the United States

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HYGIENIC LABORATORY.—BULLETIN NO. 65

JUNE, 1910

FACTS AND PROBLEMS OF RABIES

By

A. M. STIMSON

(SECOND EDITION)

WASHINGTON
GOVERNMENT PRINTING OFFICE
1912

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FACTS AND PROBLEMS OF RABIES.[a]

By A. M. Stimson,

Passed Assistant Surgeon, United States Public Health and Marine-Hospital Service, Washington, D. C.[b]

INTRODUCTION.

There has existed since ancient times a disease which, on account of its peculiar method of transmission, its unusually long period of incubation or latency after exposure, the violent and terrifying symptoms, the extreme suffering endured by its victims, and its uniformly fatal termination, has come, in spite of its comparative rarity, to be widely known to the general public and to be extensively investigated by scientists. At the same time there has been handed down from the ages when superstition and ignorance had as yet received but little counterbalancing check from the influence of reasonable or scientific investigation, a mass of erroneous belief which, in its extent and fantastic distortions, probably exceeds that to which any other malady has fallen heir.

Nomenclature.—This disease has been given various names indicating some real or fancied feature of prominence. These names may be classified as follows:

Language.	Meaning madness.	Indicating canine association.	Meaning fear of water.
Greek	λύσσα, λύττα	κυνόλυσσος	ὑδροφοβία.
Latin	Rabies, furor	Rabies canina	Aquifuga, horror aquæ.
French	Rage		
German	Wuth, Tollwuth, Lyssa.	Hundswuth	Wasserscheu.
English	Rabies, madness	Canine madness	Hydrophobia.
Italian	Rabbia		Idrofobia.
Spanish	Rabia		
Hollandish		Hondsdollheid	

Other names have been suggested, but little used. The Latin "rabies" would seem to be the best general term for the disease in both man and animals.

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[a] Submitted for publication May 18, 1910.

[b] Dr. Walter D. Cannon assisted in the work of translation and preparation of the bibliography, and the author desires to make grateful acknowledgment of his help.

Importance.—Compared with the great scourges from which man has suffered, such as tuberculosis, smallpox, plague, cholera, and yellow fever, rabies has played a very small part in the mortality of the human race; but it has annually claimed a number of victims, it has caused much suffering and anxiety in those exposed persons who have feared its onset, and it has entailed a very significant monetary loss from the death of valuable domesticated animals. If this disease were among those for which the most intelligently directed and efficiently executed measures of suppression which man can devise have proved thus far of little avail, it would be of less relative importance than even its comparative incidence would indicate. But since it has been demonstrated that, by comparatively simple measures, the disease can be reduced to a minimum or even wholly eradicated, it is raised to an importance disproportionately great to the frequency of its occurrence compared with that of less remediable maladies. The continued existence, not to say increase, of rabies in animals and man is a reproach upon the efficiency of organized medical bodies for the suppression of disease.

Study of rabies.—The fact that rabies is a well-defined entity as a disease has led to its extensive investigation from the standpoint of a purely scientific interest, as well as from that of preventive and curative medicine. And the great extent to which it has been studied is indicated by the fact that several thousands of articles on various phases of the subject have been collected from the archives of medical literature.

The literature upon the subject can, of course, be found in the Index Catalogue of the Surgeon-General's Library and the Index Medicus, but excellent bibliographies covering the most important publications will be found in the following: Högyes (49), E. Marx (69), Marie (68), Heller (45).

The Surgeon-General of the United States Public Health and Marine-Hospital Service frequently receives inquiries concerning rabies, which it is hoped may for the most part be answered in this publication.

There still remain persons who are skeptical concerning, or even deny the existence of, rabies as a distinct disease. No attempt will be made to enter into controversy with them. If they are fair-minded and possess an average amount of intelligence, a visit to a laboratory where scientific work in this direction is being done will suffice to dispel their objections even if their faith in human nature is so slight that they are able to regard those investigators who have devoted years of disinterested study to the subject as untruthful or very badly mistaken.

A BRIEF DESCRIPTION OF THE SALIENT FEATURES OF RABIES.

Before taking up in detail the study of the phenomena of rabies it is well to review briefly the salient features which combine to distinguish it.

Rabies is an ancient and widespread disorder perpetuated among the lower animals, chiefly the dog family, transmitted in nature to other animals and to man by the inoculation of virulent saliva through bites. It is undoubtedly caused by a living microorganism, as yet not positively identified, which invades the nervous system, rendering it virulent and inducing the symptoms. The period of incubation is variable, but relatively long, usually between one and two months. The symptoms are referable to excitation and finally depression of the central nervous system by the microorganism or its products, with ultimate destruction of its functionating power. A general increase of reflex excitability is early noted. The centers for respiration and deglutition are especially severely attacked, with spasm or paralysis as a result. General convulsions of central origin frequently occur, paralysis of spinal origin may attack the extremities. The psychical functions are disturbed in degrees varying in their manifestations from morbid anxiety to delirium and mania. Pathological changes of a characteristic nature are confined principally to the nervous system. The disease is essentially the same in all of the many species of animals that are susceptible. Protection is afforded by inoculation with attenuated rabies virus and to some extent by antirabic serum and, so far as known, by no other means. The blood serum of immunized persons and animals possesses specific properties against rabies virus. Once developed, the disease is almost uniformly fatal. No other disease presents these characters in combination.

HISTORY OF RABIES.

No detailed discussion of this phase of the subject is intended here. The references cited are well known and inserted merely to illustrate the ancient existence and recognition of the disease.

The earliest reference to rabies is said to be that of Aristotle in the fourth century, B. C., or possibly Democritus in the fifth century. Hippocrates, who preceded Aristotle by about a half century, is said to have made no mention of the disease. It is natural, however, that in those days of very crude methods of diagnosis, the disease was confused with other nervous affections and that because of its long period of incubation, its outbreak in man was not connected with injuries received from animals. Other ancient writers mention it, but the first extended description was given by Celsus in the first century, A. D. Cælius Aurelianus and Galen in the second century gave very good descriptions.

Cælius Aurelianus, in his description of hydrophobia in man, makes mention of the following symptoms, which are sufficiently distinctive to show that he was familiar with the same disease which we recognize to-day:

> In the beginning of the attack, anxiety with no reason for it, irritability and malaise, restless movements, sleep light and disturbed, insomnia, stretching and continual gaping, and an unremitting desire to vomit, an unusual susceptibility to air, no matter how quietly the patient may have been resting, intoleration and loathing of liquids, little desire to drink. When the disease is established, there is thirst and at the same time dread of water, at first at the sight of it, later of the very sound or mention of it. This fear extends to oily preparations. The pulse is small, hard, and irregular; in certain cases there is a light fever. There are eructations; heaviness of the limbs. The diaphragm is displaced upward and the functions of the intestines suppressed. Urination becomes frequent and of small amounts. There is nervous spasm and trembling. The voice is hoarse like the bark of a dog; breathing is difficult, the body drawn up. Generalized convulsions are occasioned by the entry of persons for fear that they may be bringing water. The face and eyes are congested, the body emaciated. Pallor and sweating of the upper parts. Frequent erections and seminal emissions. The tongue is protruded, there is hiccough, bilious vomiting, frequently black, fear of drink, the hands being held before the eyes on the approach of the drinking vessel, etc.

The Arabian physicians and philosophers transmit, without significant additions, the opinions of their predecessors. The middle ages contribute nothing to our knowledge of the disease. The disease appears again in the medical writings of the fifteenth, sixteenth, and seventeenth centuries, although in the interval in other literature indisputable references were made to it. That a paralytic form occurs in man appears to have been first recognized by van Swieten (114) in 1771. Experiments on the virulence of the saliva were first successfully carried out by Zinke, 1804, on dogs and other animals, and he also showed the destructive action of phosphorus and arsenious acid on the virus.

Gruner, 1813, recommended inoculation of test animals with the saliva of suspected dogs to establish the diagnosis.

Count de Salm-Reifersheid, 1813, made numerous successful inoculations. The first actual demonstration of the identity of the disease in man and animals is accredited to Magendie and Breschet (63), who transmitted it from man to dogs through the saliva. This important demonstration is reported in a footnote, but is clear in detail.

Following Hertwig, in 1829, very many observers have conducted experiments under various conditions, which demonstrated the disease to be an infection as we now understand it, transmitted by the saliva of rabid animals through bites.

It remained for Pasteur to devise the method of subdural inoculation of material from the central nervous system, and from him the modern study of the disease may be said to date.

The importance of this means of inoculation is appreciated on reading an article like that, for instance, of Bollinger, 1875, characterized throughout by good sense, who says that inoculation (i. e., subcutaneous) would be of little value in diagnosis and only so in positive cases. Subdural inoculation is, however, very certain and remains the only reliable means of settling the diagnosis beyond doubt. A perusal of some of the literature on rabies of the period before the infectious nature of many diseases had become known, puts us in a position to understand how the mistaken hypotheses originated in the attempt to explain the paradoxical features of the disease. These questions are, to a large extent, explained by the assumption of specific infection by a living micro-organism.

The history of rabies, as shown by its literature, is extremely instructive, as showing how much and how little was known in past ages. For instance, in Krügelstein's work in 1826 we learn that the following observations had already been made, some of them centuries before: That long-haired dogs had rabies less frequently than short-haired ones, an observation easily explained by the mechanical removal of the infectious saliva by the hair; that young dogs were susceptible, a fact which has since been doubted, but is now well established; that the saliva of dogs may be virulent before the symptoms have appeared; that the fear of water is rarely observed in rabid animals; that rabid dogs are frequently more playful at first; that mad dogs are not necessarily wild eyed, nor do they always froth at the mouth or carry the tail between the legs.

Among the superstitious and erroneous beliefs anciently entertained, and in some regions reverenced to the present day, may be mentioned that of Avicenna that the little figures of dogs, in reality blood clots, passed in the urine of a hydrophobia patient after the administration of cantharides, were the cause of rabies and that their elimination in this manner would result in a cure.

The belief that the removal of the " mad worm," which proves to be a normal cartilage in the tongue, would prevent dogs from taking rabies, was honored by an edict from Frederick the Great providing for the carrying out of the measure. The same medical authority paid a large sum for the secret of a cure for rabies, which turned out to be an emulsion of " may worms " in honey.

A popular means of diagnosis at one period consisted of stripping the feathers from the breast of a living fowl and applying the denuded area to the wound. If the dog was rabid, the fowl would die; but if the fowl remained well, no anxiety need be felt.

The modern " mad stone," still believed in and patronized in portions of the United States, is composed, according to White (118), chiefly of tricalcium-phosphate. " Mad stones " are usually calculi obtained from various portions of the alimentary tract of the lower animals. Superstition holds that they adhere to poisoned wounds (dog bites, snake bites, etc.) and remove the virus, which is evidenced by the discoloration produced when the stone is subsequently boiled in milk. The belief in these " stones " is a positive danger, since they possess none of the powers attributed to them, and their employment to the exclusion of scientific means of protection has been the cause of many deaths (Brawner).

The spontaneous origin of rabies in the dog was held in some quarters up to comparatively recent years and is still a popular belief, and various causes now disproved were assigned for it, for example: Deprivation of animal food in dogs accustomed to meat diet, ungratified sexual instinct in male dogs, sudden suppression of lactation in female dogs, extremes of temperature, especially heat, eating of putrified materials, or drinking foul water.

The chief stumbling block seems to have been this belief in spontaneous origin, which held in check experimental observations and discouraged the institution of preventive measures. The demonstration that rabies is an infectious disease, dependent for its outbreak on preceding infection, has opened up an inexhaustible field for experimental research and rendered possible those preventive measures which are effective if properly carried out.

The criticism has been made of general articles upon rabies that they exhibit a great similarity, one critic claiming that the informaton contained in them had its common source in van Swieten's commentaries on the writings of Boerhaeve. It is evident that true writings on any subject must resemble each other to a certain extent, and it is hoped that sufficient original observation by modern workers will be reported in these pages to dispel any suspicion that present-day writers are beholden to past centuries for the bulk of their information.

A perspective view of the history of rabies may be gained from the following: Rougemont (108), Krügelstein (56), Faber (29), Marx, K. F. H. (70), Fleming (34), Högyes (48), Marx, E. (69), Bollinger (14).

GEOGRAPHICAL DISTRIBUTION AND PREVALENCE.

The general statement may be made that there is no portion of the globe where man and other terrestrial mammals can live which is not potentially capable of harboring rabies. Climatic influences never operate directly to the extinction of the disease, once it has appeared. Rabies is relatively rare in sparsely settled districts unless rabid wild animals are prevalent, as, for instance, in parts of Russia, where the disease occurs among wolves to a considerable extent. The great continents all harbor rabies to a greater or less extent. It seems to be nearly stationary in some places, in others decreasing, and in some increasing. Increasing density of population, human and canine, of an infected region results in an increase in the prevalence of rabies unless rigid measures for its check are carried out.

Rabies occurs from Greenland to the Philippine Islands, where Dudley (28) reports 158 deaths in man collected by him. The disease as it occurs in the Arctic seems not to correspond exactly with that observed elsewhere and deserves special study. Colan (22) refers to it, and interesting descriptions are given by Nansen in "Farthest North." Rabies was formerly believed not to exist in Constantinople, where the dog population is very large, but Remlinger has dispelled this belief. It is, however, relatively rare there when the dog population is considered, which is explained by the great prevalence of the paralytic type.

Australia is said to be free from the disease by Bruce and Loir (17), who ascribe this condition to the peculiar fauna and the rigid quarantine on dogs which has been practiced for many years.

There seems little doubt that different strains of virus have various degrees of pathogenicity, which may explain local differences in the disease.

Rabies in America.—Rabies was observed on this continent early in the latter half of the eighteenth century. Accounts appear in the lay press as early as 1768. It appears to have occurred first in the Northern Atlantic States, but to have spread southward within a few years. Attempts have been made from time to time to secure data approaching exactness as to its prevalence in the United States, but in the past and present condition of mortality statistics in this country no accurate results can be obtained. If this is true of man, it is still more so of the lower animals. The positive results at hand, however, indicate that nearly every State in the Union has at one time or another harbored this disease in man or animals.

An inquiry (Kerr and Stimson (51)) recently made by direction of the Surgeon-General of the Public Health and Marine-Hospital Service showed the disease to be largely confined to the eastern half of the country during 1908. It is the general opinion of observers of the disease that it is on the increase in this section. Certainly more cases are recognized; but it must be borne in mind that there has been increasing activity in state and municipal agencies in securing and examining material for diagnosis.

In the survey above referred to (51) it appeared that during 1908 only ten States or territories were free from the disease in man or animals, namely, California,[a] Idaho, Maine, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming. Reports were received of 111 deaths in man during this year, an inconsiderable number compared with the deaths from tuberculosis; but when we consider the suffering involved and that these deaths could have been for the most part prevented by intelligent action, the need of increasing activity in preventive measures is apparent.

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[a] An outbreak occurred in California in the latter part of 1909. (Month. Bull. Calif. St. Bd. Health, Nov., 1909.)

The loss of valuable live stock can not be estimated from any data which we have, but judging from the loss in certain localities, it was very considerable and enough to extend a ray of hope that activity would be stimulated by it, although the death of a few children in the same localities would not be sufficient to arouse it.

The progress of the disease in various countries is thus summarized:

In Great Britain the disease is now extinct, as the result of well applied suppressive measures. It is practically so in the Scandinavian countries.

These figures from Doebert (27) show the conditions in some other European countries:

Country.	Basis.	1902.	1903.	1904.	1905.	1906.	1907.
Prussia	Persons bitten by mad or suspected animals.	250	307	365	368	373	405
Do.	Rabid animals	534	795	945	749	614	728
Belgium	do	15	35	25	94	68	237
France	Rabid dogs	2,355	2,391	2,392	2,368	2,043	1,892
Austria	Infected towns	970	1,109	1,011	1,252	1,331	1,008
European Russia	do				3,360	4,035	5,844
Holland	Rabid animals		25	1	1	51	41
Italy	Rabid domesticated animals	428	33	329	310	792	701

The increasing strictness of inquiry must be considered in some of the above countries before an increased prevalence as indicated in the figures can be assumed. And again, it must be remembered that, while a total increase may appear, marked reductions have resulted in areas where rigid measures have been carried out.

As is the case with many other diseases, rabies may be quite prevalent in a territory without coming to official cognizance or gaining public notice unless attention is drawn to the condition by the death of some person from hydrophobia. This is well illustrated by the conditions reported by Salmon (111) in the District of Columbia, where an investigation, stimulated by such a death, revealed a quite unsuspected prevalence among dogs. We believe that there are comparatively few cities in the eastern portion of the United States where a careful investigation extending over a year's time would fail to reveal the presence of rabies in dogs.

ANIMALS AFFECTED AND SUSCEPTIBLE.

While nearly or quite all warm-blooded animals are susceptible under favorable conditions to experimental inoculation of the virus of rabies, it is more commonly met with in nature in those mammalians which are subjected most often to the bites of dogs, wolves, coyotes, etc. Some species are relatively seldom infected because they are seldom subjected to bites or because they are provided with a thick fur which tends to mechanically prevent the introduction of the virus, or possibly because of some natural relative immunity.

There is some reason to believe that skunks may propagate the disease widely among their own kind as the dog does, and cases are know of infection of man by this animal; but as a rule animals other than the dog or his kind do not transmit the disease another remove. This may be explained by their relative lack of ability, opportunity, and tendency to bite when rabid.

The demonstrated susceptibility of rats and other rodents to subcutaneous inoculation has led to the opinion, entertained by a few, that these animals might be capable of perpetuating rabies without the mediation of the dog. Their limited contact with man, however, argues little danger from this source, and the success of the measures directed against the dog alone, as for instance in England, shows that this fear is unfounded. It has been shown that rats may acquire fixed virus infection by ingestion of rabic brains. Perpetuation of the disease in this manner among animals dangerous to man seems far from probable.

The domesticated animals are affected with something like the following relative frequency, subject, of course, to variation according to conditions:

Animals sickened and died, or killed on account of rabies in Prussia.

Animals.	Doebert's figures, 1902–1907.		Schüder's figures, 1886.	
	Number.	Percentage.	Number.	Percentage.
Dogs	3,715	85.1		80.2
Cattle	467	10.7		14.7
Horses	63	1.44		1.0
Swine	49	1.12		1.6
Cats	35	.81		.7
Sheep	32	.7		1.7
Goats	4	.09		.1
Other animals				.02
Total	4,365		11,306	

Biting animals rabid or supposedly rabid, in cases from Paris and Budapest institutes.

[From table by Högyes.]

Animals.	Paris percentages.	Budapest percentages.	Animals.	Paris percentages.	Budapest percentages.
Dogs	93.13	90.32	Man	0.04	0.26
Cats	5.75	7.80	Jackals	.03	
Cattle	.37	.52	Sheep	.02	
Horses	.22	.28	Foxes	.02	.02
Asses and mules	.18	.06	Rabbits		.02
Wolves	.12	.38			
Swine	.07	.32	Total number of cases	14,296	4,961

Considering now the animals which have been successfully inoculated experimentally, it may be stated that all mammals so far tried (a large number of species) have proved capable of taking the disease. Birds also are susceptible of inoculation, but seem to have a relative immunity, as old fowls and pigeons if they take the disease at all frequently recover and their nervous system is innocuous to rabbits, although it may transmit the disease to other fowls. This relative immunity is possibly due to their high body temperature, 108° F.

Frogs are said by von Lote to be susceptible, but to exhibit such a long incubation period that many experiments have been reported negatively because not continued long enough. Marie also found that frogs are susceptible; but Dammann and Hasenkamp, and Galli-Valerio obtained only negative results.

Remlinger states that the tortoise is refractory to rabies, an observation confirmed by Carowgean (20).

SEASONAL PREVALENCE.

The disproportionate prevalence of rabies during certain seasons of the year, long a matter of belief, seems to have little foundation in fact. What foundation it may have is either a matter of local coincidence or is due to the fact that more men and animals are likely to be abroad and liable to meet with infections at some seasons, notably the warm months, than at others, and not to any increased susceptibility.

The following statistics show that the seasonal variations are not marked or constant in any direction, except that the last quarter of the year usually shows relatively fewer cases:

Based on—	Author.	Place.	Years.	Jan.	Feb.	Mar.	Apr.	May.	June.
Rabid animals	Schüder	Germany	1886–1901	24.4 per cent.			27.6 per cent.		
Rabid dogs	do	France	1897–1901	24.2 per cent.			27.4 per cent.		
Persons treated	do	Berlin	1898–1902	25.9 per cent.			26.7 per cent.		
Persons bitten	Doebert	Prussia	1907	25.8 per cent.			25.5 per cent.		
				168	171	194	197	186	144
Rabid dogs	do	France	1903–1907	24.8 per cent.			30.2 per cent.		
				773	866	1,051	1,086	1,035	1,153
Persons bitten	Blatchford	United States.	To 1856.[a]	33.3 per cent.			25.3 per cent.		
Rabies in dogs	Salmon	Various		20.9 per cent.			29.2 per cent.		
				943	1,045	960	1,323	1,419	1,467
Total average				25.0 per cent.			27.4 per cent.		

Based on—	Author.	Place.	Years.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Rabid animals	Schüder	Germany	1886–1901	25.5 per cent.			22.5 per cent.		
Rabid dogs	do	France	1897–1901	25.4 per cent.			23.0 per cent.		
Persons treated	do	Berlin	1898–1902	26.5 per cent.			20.9 per cent.		
Persons bitten	Doebert	Prussia	1907	22.8 per cent.			25.7 per cent.		
				171	148	152	226	150	155
Rabid dogs	do	France	1903–1907	24.8 per cent.			20.4 per cent.		
				1,044	807	802	782	728	704
Persons bitten	Blatchford	United States.	To 1856.[a]	24.3 per cent.			15.3 per cent.		
Rabies in dogs	Salmon	Various		27.5 per cent.			21.5 per cent.		
				1,435	1,294	1,145	965	933	1,137
Total average				25.2 per cent.			21.3 per cent.		

[a] 101 cases.

PATHOLOGY AND ETIOLOGY.

The symptoms of rabies all point to a disturbance of the nervous system, and the anatomical changes abundantly confirm the idea that this is the part of the body primarily affected. Certain glandular organs share in the affection to the extent of disturbance of function and of containing the infecting agent. That the disease is an infectious one, i. e., produced by the invasion of a pathogenic microorganism emanating from a previous case of the diseases, is strongly suggested by the symptoms and the manner of conveyance, and is proved by the fact that all the parts of the nervous system, as well as some glandular structures and secretions, produce the same symptoms and results when inoculated experimentally into a healthy animal.

The most generally accepted view as to the manner of the pathogenesis of the microorganism causing rabies is that upon its introduction beneath the epidermis or mucosa it finds its most favorable or perhaps its only favorable medium for propagation in the nerve endings or torn fibers of the region. Along the course of these it develops, not disturbing their function although rendering them infective, until the central nervous system is reached. Here the nerve cells are attacked, the first effect being a hyperstimulation of their activity, the final result a destruction. At the same time, as certain authorities maintain, there occurs the production of a toxin, extra or intracellular, which is responsible for some of the symptoms. This latter view is opposed by others, and the elucidation of the question constitutes an important problem with a bearing on methods of treatment.

There is good experimental evidence that the explanation as above briefly outlined is correct. Nerves leading up from the site of the inoculation to the central nervous system have been shown to become progressively infectious in ascending segments, while their section previous to inoculation confines the infectious agent to the lower segment. The blood and lymph streams seem to be almost entirely incapable of taking up the microorganism from the site of inoculation and presenting it in a pathogenic condition to the cells of the brain and the cord. The blood of rabic animals has usually been found nonpathogenic when directly inoculated, but a few successful inoculations are recorded (Marie (64), Koch, J. (52)).

If we except for the time being the Negri bodies, the anatomical changes, gross and microscopic, can not be said to be characteristic of and confined to rabies, as they have been found in other conditions. They are, however, for the most part fairly constant and may be considered as directly connected with the disease in a specific manner. Many of them are produced during the prolonged death agony and are consequently not peculiar to this disease. To review these changes:

Meninges.—These are usually congested and the vessels stand out in a very evidently dilated manner. There are punctate hemorrhages here and there over the surface. These changes may be mechanical and due to the convulsive seizures, although they are found in the paralytic type of the disease where generalized spasms are infrequent. The meninges are frequently edematous.

The *cerebrospinal fluid* is said to be increased in amount and consequently under high pressure.

The *brain* and *cord* show the same vascular changes to a less extent than the meninges. Areas of softening have been observed, visible sometimes to the naked eye, but often microscopic in size. A perivascular infiltration with lymphocytes and embryonal cells may be noted throughout the central nervous system, and cellular proliferation in the vascular tunics, even to the extent of occlusion, is described. These changes are similar to those observed in progessive paralysis and sleeping sickness (Achúcarro (1)). An infiltration with embryonal elements is observed around the motor nuclei of the bulb and cord, constituting the "rabic tubercles" of Babes (2).

The nerve cells themselves, especially in certain regions, may show marked changes, those in the interior of the "rabic tubercles" being degenerated.

In the *peripheral ganglia*, especially the pneumogastric fusiform ganglia, the cells are in a majority of the cases much degenerated, the capsules in which they lie are thickened through proliferation of their cellular elements, and the connective tissue is infiltrated with round cells. This condition is sufficiently constant and characteristic to be of considerable diagnostic importance, although not conclusive. The nerve cells in rabies are usually degenerated in some portion of the brain and not in others. The changes seen are irregular distribution of the chromatophilic elements, vacuolization, shrinkage of the cell as a whole, and eccentric disposition of the degenerated nucleus. Changes in the neuro-fibrillar network have been shown by Cajal's method. The brain cells in rabies are frequently not found markedly degenerated when the coarser histologic methods are used in routine examinations.

The great majority of observers are agreed upon the specificity of the changes described by Negri, but there is a division of opinion as to whether they are the parasites themselves which cause the disease, whether they contain the much smaller parasite in their interiors, or whether they represent a specific reaction of the nerve cells to the poison. They consist of sharply defined round or oval structures occurring within the nerve cells of the brain and cord, taking the stain in a manner very distinct from the cell structures, and containing minute granules which stain differently from the rest of the body. They are very constantly found in rabies at all stages of the disease after the onset of the symptoms, and have a predilection for certain portions of the brain, although found at times in nearly all portions of the central axis. They have been described by Stefanescu in the parotid gland of one dog dead of rabies. (See under Diagnosis, p. 34.)

Outside of the nervous system there are few changes not secondary to its involvement. The fauces, pharynx, and larynx are usually deeply congested. The tongue (in dogs) is dry and may be blackened. The salivary glands are inflamed and their secretion before death is much increased. Retention cysts under the tongue of carnivorous animals may sometimes be formed, due to the undue activity of the glands and to swelling and occlusion by desquamated epithelium of the ducts.

One of the exploded hypotheses of rabies is that of Maroschetti, who claimed that these "vesicles" appeared in man before the development of rabies; that the virus was contained in them, and that its liberation by incision followed by cauterization was a sure preventive of hydrophobia. The pancreas, adrenals, spleen, and kidneys show engorgement, as may other glandular structures. Babes and Jonnesco (5) have recently described at some length the lesions of the pancreas and the salivary glands.

In the lower animals, especially dogs, the gastro-intestinal tract is frequently devoid of normal contents, food or chyme, and instead are found a variety of unusual foreign bodies—sticks, stones, straw, splinters of wood, feathers, etc. This constitutes a finding of doubtful diagnostic value, as it is known that dogs suffering from diseases other than rabies, sometimes swallow such objects, as do also wandering dogs when suffering from hunger; but it was relied upon before subdural inoculations became depended upon for making the diagnosis certain.

It must be admitted that, with the exception of the Negri bodies, the microscopic anatomy of rabies offers nothing which is constant or characteristic, although the changes above briefly described are met with in a majority of the cases. There is one, however, not demonstrated by the microscope, which is absolutely constant for the nervous system and frequently present in certain of the glands, chiefly the salivary, and is absolutely characteristic. This is the acquisition of the property of causing rabies in fresh animals when suitably inoculated. For a discussion of the findings of different observers of the pathological anatomy of rabies, see Högyes (49), Remlinger (96), Marie (68).

The *blood* is stated by Courmont and Lesieur (23) to present from the early stages until after death a relative polynucleosis which is constantly found. The lung juice also presents this condition. Other observers describe leucocytosis.

The *urine* sometimes contains albumin, but glycosuria is a more striking phenomenon, although apparently uncommon in man. Acetonuria sometimes occurs.

RABIES VIRUS.

As ordinarily used this term now applies almost exclusively to the virulent nervous substance of an animal suffering or dead from rabies. It comprises the specific cause of the disease and the medium in which it is found, i. e., the nervous substance. The nature and properties of the virus will be discussed under the subject of *immunity*, but it may be well to point out here certain differences between "street" and "fixed" virus, as they will be frequently referred to in the following pages.

Street virus (virus des rues, Strassenvirus) is the virulent nervous tissue as met with in the natural disease. Its virulence is very variable and when inoculated into rabbits subdurally it causes the symptoms of rabies to appear at a variable period of more than fourteen days as a rule. There have been exceptions to this noted in several instances when the incubation was as low as eight days. Instances of this kind are collected from the literature by Nitsch (79). Consequently the incubation period can not be taken as an absolute criterion as to whether a given virus is of natural or laboratory origin. This question is sometimes raised when a person has died of rabies after having taken the Pasteur treatment. It has been suggested that dogs are more reliable test animals for this purpose than are rabbits. Again the inoculations should be carried on for several passages when the nature of the virus should become clear.

Fixed virus (virus fixe) is modified from the street virus by passing it through a long series of rabbits. In this way its virulence becomes greater for these animals, so that they finally take the disease after a constant "fixed" period of incubation. No amount of passage can reduce the incubation period below this point, so the virulence is said to be fixed. Other considerations in this connection will be taken up later.

INCUBATION PERIOD.

The incubation period of rabies is remarkable for its length and variability. Its length is in nature seldom under ten days, and may extend over many months. It is difficult to say what the longest period has been; but it is generally admitted that a year or more is not impossible. The majority of cases occur before the end of the third month. Extremely long periods as reported always lead to the suspicion of a mistake or the occurrence of an unobserved or forgotten intermediate exposure. The fact that the saliva of animals may possess infective properties several days before the outbreak of significant symptoms may explain the occurrence of unsuspected infections within the supposed incubation period, since trifling bites or licking of abraded surfaces by an apparently healthy dog would be likely to escape attention or be forgotten.

The length of incubation is influenced by certain known factors:

(1) The species of animal affected. From a review of the statistics on this subject it would appear to be longer in man than in the lower animals, and among them, in a general way, proportionate to the size of the animal.

(2) The site of inoculation. The shorter period in cases of bites near the brain accords with the accepted views on the transmission of the virus along nerve trunks. The shorter the distance to be traversed the shorter will be the period of latency, other factors being equal.

(3) Sex. Females, at least in the human species, exhibit a shorter period than males.

(4) Children usually have a shorter period of incubation than adults, but this may be because bites in children are frequently severe and about the head.

(5) Severity of wound. Wounds in which nerve trunks or muscles are torn result in a relatively short period of incubation, both because of the large amount of virus introduced and because it is brought into immediate contact with the nerve tissue.

(6) It is probably true that early onset of the disease may be induced by certain causes which tend to weaken the resistance of the nervous system. Alcoholic indulgences, sudden shocks as from cold baths or falling into the water, excessive fright or other nervous excitement, and certain diseases affecting the nervous system, e. g., syphilis, antecedent meningitis, have in many instances seemed to determine an early onset.

(7) Treatment. Nitsch has pointed out that in a large series of cases the deaths, in spite of the Pasteur treatment, occurred on an average earlier than in untreated persons (sixty-four and one-half days to ninety days), and that this would naturally be expected when the rationale of the treatment is understood, since cases in which the incubation would have been long, if untreated, are those which afford the most time for the establishment of immunity if treated.

(8) There is some reason to believe that rabies virus, as it occurs in nature, varies much in virulence, and that this is in some way related to the geographical distribution. Thus, the short incubation periods of the cases reported in Egypt by Bain and Maloney may have relation to an especially potent virus in this region, as compared with that commonly prevalent in Europe.

During the period of incubation no unusual symptoms are complained of. The wound heals as other wounds do without any specific phenomena. Some persons are very anxious about the outcome, and may get into a condition of nervous excitement; but they are as likely to do so after harmless as after infected wounds.

A general idea of the duration of the period of incubation in man and various animals may be gained from the following statistics and figures:

Incubation period in man.—Bauer (11), in 1886, published the results of an inquiry into the incubation period of 537 cases of human rabies, representing all the material available to him at that time. Seventeen of these cases had an incubation period of over one and one-fourth years, and 10 of the remaining cases were regarded as doubtful. The average period in the remaining 510 cases was seventy-two days. In *males* the average was eighty days, in *females* sixty-five days. The period was influenced by *age* as follows:

Number of persons.	Age.	Average period.
	Years.	*Days.*
120	2 to 14	57.0
182	15 to 50	77.5
45	51 to 78	70.0

The location of the bite was determined in 252 cases, the average periods being—

	Days.
For 73 cases of head and neck bites	55
For 3 cases of buttock bites	26
For 144 cases of upper extremity bites	81½
For 17 cases of lower extremity bites	74
For 15 cases of bites on several parts of body, many being on the head	55

Bites on the buttocks are not ordinarily followed by as short a period as indicated.

According to the biting animal, modifying virulence and severity of bite—

	Days.		Days.
49 wolf bites	39	2 fox bites	33
293 dog bites	73½	1 cow bite	30
31 cat bites	80		

No effect of local treatment was noted, nor any relation between the incubation period and the duration of illness.

The percentage of Bauer's total number of cases developing rabies at specified periods—

	Per cent.		Per cent.
1 to 19 days	8.24	100 to 149 days	7.65
20 to 39 days	28.43	150 to 199 days	5.69
40 to 59 days	21.18	200 to 249 days	.98
60 to 79 days	15.30	250 to 330 days	2.35
80 to 99 days	9.22	1 to 1¼ years	1.18

Incubation period in animals.—Friedberger and Frohner (35) give the following figures for the incubation in animals:

Ox, 4 to 8 weeks average. Shortest, 1 week; longest, 2 years.

Horse, 4 to 8 weeks average. Longest, 12 weeks; 2 cases—283 days, 20 months.

Cat, 2 to 4 weeks average.

Pig, 2 to 3 weeks average. Shortest, 6 days; longest, 179 days.

Sheep, 3 to 4 weeks.

Poultry, 6 weeks to 11 months.

Nocard and Leclainche (82) give the following figures:

Dog and cat, 15 to 60 days average. Shortest, 8 days; longest, 1 year.

Horses, 15 to 60 days average. Cases as high as 10, 14, and 20 months (four-fifths of cases under 60 days).

Cows, 1 to 3 months average. Cases as high as 20 and 23 months.

Sheep and goats, 15 to 30 days average.

Swine, 15 to 30 days average. Extremes 6 days (?); 6 months.

Högyes says, concerning the incubation period in dogs, that 60 days' average is usually given, but that under experimental conditions at Budapest it is considerably shorter, e. g.:

	Days.
By bites	9 to 33
By subcutaneous infection	13 to 19
By subdural infection	10 to 15

SYMPTOMS AND CLINICAL COURSE.

Rabies presents at least two clinical types. These are the furious or excited, and the quiet, silent or paralytic. Paviot and Lesieur (88), distinguish, according to the portion of the nervous system most involved, four types of the disease: Cerebral, medullary, cerebellar, and sympathetic.

The study upon which this classification was made was very thorough, but embraced too few cases to generalize from. The authors apparently trace a connection between the type of clinical manifestations and the localization of the most pronounced lesions.

These variations, however, are all within the theoretical limits of a specific involvement of the nervous system by a microorganism or its products. The disease is essentially the same in all animals, although there is a predominance of certain symptoms or of one form or another in different species.

The excited or furious type in man.—The onset of rabies is usually rapid. The patient usually shows some psychical change very early, becoming anxious, melancholy, and oppressed with a strange presentiment of harm. Insomnia is complained of. Such symptoms, however, are in many cases absent at first, and local numbness, tingling, itching, and formication in and progressing centrally from the wound may be the only disturbance. The wound or scar may become somewhat engorged and tender. Sometimes the first symptom complained of is a strange sensation in the throat, difficulty or discomfort in swallowing, a sense of constriction of the fauces.

Bain and Maloney (7) record a case in which the first symptom was difficulty in walking, and another in which cardiac irregularity was the first sign. There are many cases recorded where the onset was apparently determined by some severe mental shock, and the initial symptoms may be apparently purely hysterical. In fact, hysterical manifestations are not unusual and may lead to a favorable diagnosis and prognosis. These symptoms are in some of the cases part of the psychological pathology of rabies, an aggravation of the usually less violent aberrations. The state of mind induced by the beginning disease often renders the patient susceptible to excitement on exposure to impressions which ordinarily would not produce it. Fright and terror may, therefore, be regarded in many instances as a manifestation of the disease, not as the determining cause of its outbreak. It is conceivable, however, that any influence causing over excitement of the nervous system with subsequent fatigue or exhaustion might predispose to the more rapid invasion of a microorganism, or increase the susceptibility to a toxin. An initial rise of temperature is perhaps the most constant early sign, the elevation being moderate as a rule.

The symptoms usually progress without delay after the preliminary signs are observed. The forewarning symptoms may last several days before the decided outbreak, but usually only twenty-four to forty-eight hours. The "grand symptom," hydrophobia, is present in the majority of cases, although influenced by the patient's disposition and surroundings to a considerable extent. It arises from the extremely painful spasms of the organs of deglutition and respiration, which are induced by attempts to eat or especially to drink. These spasms are often of such an agonizing character that the thought of them causes a mental anguish not exceeded in the possibilities of human suffering of physical origin. Consequently the sight, smell, or sound of liquids suggests the act of swallowing and is sufficient to bring on an attack in many cases. The patient, if a person of exceptional self-control, may sometimes be diverted from the subject for a considerable time and be free from the painful seizures. Sometimes the patient goes through an elaborate formula in preparing to drink, like a child insisting upon a special arrangement of all accessories in an effort to protract the time before taking an unpleasant dose of medicine. Then an effort is made to gulp down a swallow of the liquid only to have it forcibly expelled with an accompanying anguishing spasm of the throat and larynx.

The condition consists, therefore, of a hypersusceptibility of the nerve cells to external stimuli. If these stimuli can be removed, cell action may be kept temporarily in abeyance. The cell explosions may be evoked by phychical stimuli and through the special senses. Drafts of air, lights, sounds, and even smells, may bring on a convulsive seizure. Skin and tendon reflexes are exaggerated. The respiratory spasm involves the thoracic muscles and can not be relieved by intubation. In fact, the glottis may be open during an attack. Solid food is usually more readily taken than are the fluids.

Whatever the onset the disease usually progresses rapidly. In a minority of cases there may be periods of absence of symptoms which cause the hope of recovery and doubts of the diagnosis to be entertained. Remissions always occur except in the extremely rapid cases. As the disease progresses the symptoms become more severe. The mind is usually clear, questions being answered with understanding until the voice becomes indistinct and the words unintelligible. In a large number of cases the patient is very talkative, speaking incessantly about himself and his disease until interrupted by a spasm.

There are periods of excitement which may be truly maniacal, the patient raging about the room, destroying furniture, and trying to escape. There is seldom, however, any tendency to injure other persons, much less to bite them as popularly supposed. After a violent period the patient may realize his acts and be sorry for them. He frequently seems to know when an attack is coming on, and begs to be restrained lest he injure someone. Sexual excitement, accompanied by priapism, is a frequent symptom. The voice becomes hoarse with a peculiar quality. The strange sounds emitted during expectoration or on the onset of the seizure have given rise to the popular statement that the patient "barks like a dog."

The convulsive seizures become more frequent and severe and distributed over a larger area. Sometimes the muscular contractions are so severe as to cause rupture of the muscles. Small hemorrhages from mucous surfaces and elsewhere may occur. Vomiting is a rather frequent symptom, and the vomitus is often black. The color is said to be due to regurgitated bile, but blood also is sometimes found.

Death may occur during the convulsive stage, but more frequently a condition of paralysis mercifully leads the patient on to death. The spasmodic seizures become less severe and frequent and less readily evoked. The muscles, racked to the limit of human endurance, become limp. The face, which had expressed terror and extreme suffering, becomes smooth and expressionless. The jaw drops and the mouth hangs open. There is commonly an excessive secretion of saliva of a ropy character which the patient is unable to expel and which flows out of the corner of the mouth in large quantities; but where this is not abundant, the tongue becomes dry and hard. The breathing becomes irregular and feeble, and finally stops. The temperature ascends before, and it is said immediately after death in some cases; but where this stage is prolonged the temperature is more likely to be subnormal. Sugar and acetone are sometimes found in the urine, although not as constantly in man as in some of the lower animals, especially the herbivores.

The eye symptoms described are photophobia, mystagmus, and sometimes strabismus. In the paralytic stage the pupil is dilated from paralysis of the iris.

In a general way, then, the symptoms may be grouped into three stages: That of prodromata, the excited stage, and the paralytic. They often merge into each other, so that their limits are uncertain.

The paralytic type in man.—This was early described by van Swieten. Because its symptoms are less marked than the violent type it has undoubtedly been unrecognized in many cases, and the disease has been incorrectly diagnosed. In fact, its existence seems to have been lost sight of for a time.

In 1887 Gamaleia (39) caused renewed interest to be taken in the matter by his publication of a number of cases taken from medical literature and his own observations, and it must now be regarded as a rather common form of the disease in man, as it is undoubtedly in dogs and other animals. Pathologically it has been attributed to infection with a large amount of virus and to involvment of the spinal cord rather than the brain. The symptoms are those of the last stage of the excited form, without any pronounced antecedent symptoms of excitement. They bespeak a transitional form between the paralytic rabies of the smaller animals and the convulsive form of hydrophobia in man. Gamaleia sums up the manifestations in the cases reviewed by him as follows:

Onset with high fever, general malaise, cramps, headache, and vomiting much as in the ordinary convulsive form. Afterwards, localized pains, ordinarily in the bitten parts, but rarely so when these are in the lower extremities. A heaviness and numbness of these parts follow, then ataxia and weakness followed by more or less complete paralysis. A girdle sensation is frequently complained of. Anaesthesia is uncommon. Consciousness is ordinarily retained, at least until late in the disease.

The paralysis spreads with preceding or accompanying pains of the affected parts, invading the limbs, trunk, rectum and bladder, face, tongue, and eye muscles. Respiratory involvement is variable in the time of its appearance and severity. Inspiration is more affected than expiration. Dyspnoic convulsions may result when the condition is severe.

More or less difficulty in swallowing liquids results from the respiratory embarrassment, but the symptom "hydrophobia" is caused more by the imagination than by the disease. Frequently normal respiration may be restored for a time. Death occurs by cardiac paralysis, according to this author.

This form of the disease is more prolonged than the furious type, lasting up to seven and one-half days, while in the latter the average duration is three or four days.

RABIES IN THE LOWER ANIMALS.

Rabies is the same disease pathologically and clinically in whatever animal it occurs. It is true, however, that one clinical type or the other predominates, according to the species affected, and that different animals have various ways of showing the symptoms.

The dog is almost an intellectual anomaly among animals. By centuries of association with man, of a kind more intimate than any other enjoys, it has acquired a mental development far in advance of most of the domesticated animals. It is natural that psychical disturbances should be marked in this animal. When a dog shows a marked and apparently causeless change of its habitual disposition, suspicion of rabic infection should be entertained, especially if an opportunity for such is known to have occurred. This change may be either in the direction of becoming more morose, sullen, and irritable, or of showing unusual affection and desiring to be petted more than usual. Fatal infections of man have occurred during this stage through licking of abraded or scratched surfaces by an over-affectionate animal, or by bites received in a period of unnatural playfulness.

A very characteristic symptom is the change in the character of the voice. It has been aptly described as resembling the belling of a tired hound which has been chasing game for a long time. Instead of the normal succession of sharp barks, there is a hoarse howl followed by an unequal series of barks, lower in pitch than is normal, the jaws not being completely closed between them as is normally the case.

The rabic dog, whatever his initial symptoms, soon becomes restless, lying down and getting up again repeatedly. He is easily startled, and growls and barks on slight provocation. In the furious type, after a few days or less of inquietude, he may suddenly leave home, wandering off for many miles perhaps, to return in a day or two, emaciated, wounded, and utterly changed. During the period of "running mad" he may have bitten many persons and other animals. He may bite and tear his own flesh at the site of the wound, and seems impelled to bite anything that comes in his way, as if some relief could be gained in that way. He can not be said, as a rule, to fight with other dogs. He bites them and passes on, but does not go out of his way very much to attack them. His main object seems to be to keep moving, as if to escape something.

The popular idea of a mad dog is a raging animal with glaring red eyes, the froth flying from his jaws, and the tail carried between the legs. A picture of such a dog appears as the frontispiece to Rougemont's treatise on rabies (1798). This is an unusual picture in actual experience. The dog looks sick, takes no interest in his surroundings, but trots ahead, perhaps with wavering gait. The conjunctivæ are, it is true, usually injected, but frequently not enough to attract attention, and the tail may be carried over the back. It is the rule that dogs in this condition have no appetite for their accustomed food and frequently swallow indigestible objects, such as sticks, stones, straw, etc. Swallowing is difficult and, later in the disease, impossible.

Convulsions of greater or less distribution now appear, and the animal may die in one. More frequently a paralytic stage supervenes. The dog drags himself to a secluded place. The hind legs are usually paralyzed first. It is often thought by the observers that his spine has been injured. The jaw drops from paralysis of its muscles. The tongue is apt to be dry and may be blackened. A black discoloration of the roof of the mouth is no criterion whatever for rabies, as it occurs in healthy dogs. Salivation may be profuse, the saliva drooling from the mouth. This is not incompatible with the dried condition of the tongue. The animal becomes much emaciated. Death is caused by the ascent of the paralysis to the respiratory centers.

The *paralytic form* is quite frequent among dogs and offers peculiar danger to man. These are the cases where the owner or sympathetic bystander endeavors to remove an imaginary bone from the throat and becomes bitten or scratched. Spasms of deglutition and the animal's actions during them, and paralysis of the throat muscles, strongly suggest an obstruction in the throat. It is a mistake to suppose that rabid dogs have any fear of water. They are intensely thirsty and desire to drink, but when the paralytic condition affects the throat muscles they are unable to swallow. They are so far from having a fear of water that they sometimes swim rivers while in the furious stage, as observed in ancient and modern times. Young puppies have been thought to enjoy an immunity to rabies, but this has been shown to be incorrect by Remlinger (103).

Glycosuria as a symptom of rabies has been described by Nocard, Rabieaux and Nicolas (95), and others. It is stated to be very constant in herbivores, less so in carnivorous animals. It is not of renal origin, since the blood contains sugar in excess.

A detailed account of the symptoms of natural rabies in other animals does not seem to be called for, as they are but modifications of type or intensity of the disease in dogs. The paralytic form is very frequently in herbivores, but horses frequently have the furious type in its most agonizing form. Fowls are said to have the furious form by Friedberger and Frohner (35), but other observers are almost unanimous in describing a slow paralysis in them, both the incubation and duration being prolonged.

Experimental rabies in the rabbit requires special description, because it is this animal which is now most used for diagnostic inoculations of material from man, dogs, or other animals.

After subdural inoculation the animal evidences no symptoms whatever for several days to two or three weeks, depending on the nature of the virus, i. e., fixed or street virus, virus of high or low virulence. The first sign to be noted, if the animal is examined for it, is an elevation of temperature. v. Löte (60) states that this transient premonitory fever is seen in rabbits inoculated with virus of medium virulence. The animal appears sleepy, does not eat; its movements are incoordinated and it is apt to fall. The appearance of the face is quite characteristic, the eyes having a staring expression, and frequently one ear hangs down while the other remains erect. The animal urinates much more frequently and copiously than normally, and this, coupled with the decreased consumption of food, results in a very evident emaciation. Rarely there is a brief furious stage in which the animal throws itself against the cage and tries to escape. Gritting of the teeth is frequently observed. Convulsive seizures are frequently seen, involving the legs, back, and jaws. They persist after the animal can no longer sit up and lies on its side, so that the litter in the cage is scraped in a segment of a circle where the legs move. Paralysis usually begins in the hind legs and proceeds forward, but may be reversed. The usual position in this stage is on the side with the head drawn sharply backward; anterior flexion is rare. Movements become weaker until only a feeble respiratory movement is visible and the animal dies. Abortion in pregnant females is very common.

DIAGNOSIS.

The diagnosis of rabies rests upon the consideration of as many of the following factors as are available: (1) History of exposure, (2) the length of the period of incubation, (3) the clinical symptoms and course, (4) the termination, (5) the post-mortem findings, gross and microscopic, and (6) inoculation tests upon animals. These will be considered in detail.

(1) *The history of exposure.*—While the great majority of patients suffering from rabies will give an account of being bitten or scratched by an animal, or having an abraded surface exposed to the saliva of one at some time previously, there are some cases where this is lacking. Such cases were formerly described as "spontaneous" or "idiopathic," but it is a fundamental doctrine that infectious disease does not

originate *de novo*, but is always derived from previous disease in another host. It is not surprising that in a disease like rabies, the incubation period of which often extends over many weeks, slight scratches received from dogs or other animals, or licking of the hands on which there were trifling wounds, might be forgotten at the time of onset. This is the more apt to occur since the saliva of animals has been shown experimentally to be infectious several days before the appearance of noticeable symptoms in them. Again, it sometimes happens that a patient realizing the character of the disease with which he is beset, attempts to deceive and reassure himself by denying any knowledge of infection. The evidence of other persons will often clear up such cases. It sometimes happens that no history of infection may be obtained until late in the disease or after death, when some acquaintance furnishes the information. There are some recorded instances where the patient died apparently of rabies but where the biting animal has after a period of illness recovered. Dogs have, under experimental conditions, recovered from developed rabies, so that their recovery can not be regarded as positive proof of the nonrabic character of the disease. The fate of the animals biting or being bitten by them should be inquired into. At any rate, the history of a bite by an animal known or thought to be rabid is only suggestive and does not rule out other diseases. In animals evidence of infection is frequently lacking. Opportunity for it should be considered and scars looked for if it can be done with safety.

(2) *The length of the period of incubation.*—There are some apparently well-authenticated cases of development of rabies in man as early as ten days after exposure. This probably is the lower limit of this variable period. Hysterical manifestations simulating rabies usually come on within a few hours or days after the assumed exposures, or if appearing late are induced by seeing, hearing, or reading of some real case of the disease.

The occurrence of symptoms like those of rabies at a period less than two weeks or more than one year after the exposure should be regarded with suspicion, and every effort made to clear up the other factors bearing upon diagnosis.

The mental behavior of the patient during the period may be of assistance in making a diagnosis, or on the other hand lead to confusion. Many cases show no disturbance whatever until the unmistakable signs develop. Others from the time of the bite are in such a state of anxiety that it is difficult to tell at first whether the new symptoms are a product of hysteria or due to rabic infection.

(3) *The clinical symptoms and course.*—While the symptoms and course of rabies are usually sufficiently distinctive clinically to rest a diagnosis upon as firm a basis as obtains in other infections, such

a diagnosis nowadays should be confirmed both in man and in the lower animals by laboratory methods where these are available. It must be admitted, however, that there are occasional cases which are difficult or impossible of clinical diagnosis. Confusion may be caused by hysterical symptoms. In this connection witness the interesting case reported by Calabrese (18), in which the onset seemed to be determined by reading an account of a case of hydrophobia and the symptoms were markedly hysteroid, and a period of amelioration of the symptoms made a diagnosis of hysteria seem permissible. Death occurred, and the brain substance caused rabies in rabbits, two removes. Where these hysterical manifestations appear within a week of exposure (provided there have been no previous exposures) rabies can be excluded, so far as present experience teaches. In the condition called lyssophobia the symptoms popularly ascribed to rabies may be closely simulated. They are apt to be exaggerated and ill timed. For instance, the first signs may be the symptom hydrophobia and a good imitation of a dog's bark and to some extent its actions, the patient going about on all fours and biting at the furniture. The hysterical stigmata are present, and the patient may be cured by suggestion.

Once a patient came to the Hygienic Laboratory a few days after handling a dog thought to be mad. He complained of a sense of constriction in the throat, pains in his head, and was visibly nervous and excited. He thought himself to be in the grip of the dread malady. His symptoms subsided promptly upon being emphatically reassured. Such cases could be multiplied. It is stated that hypersensitiveness to drafts of air, which is very common and pronounced in true rabies, is not simulated in hysteria. This may be tested by stepping unobserved behind the patient and fanning him. A convulsive seizure induced in this way would serve to strengthen a diagnosis of rabies. Tetanus and mania may simulate rabies. The absence of trismus and continuous spasm serve to differentiate rabies from the former, as do also the long period of incubation and other factors mentioned in this discussion. Mania seldom takes the form of rabies, and can be differentiated by etiologic considerations. Simple throat infections with fever, pain, and difficulty in swallowing occurring after exposure to rabic infection are sometimes indistinguishable from incipient rabies; but the course of the disease will differentiate it within a short time.

It is to be emphasized that upon clinical evidence alone the diagnosis will sometimes, but not often, be uncertain. Remlinger (100) considers some cases diagnosed as Landry's paralysis as true paralytic rabies and advocates special investigation of such cases with this point in view.

In the lower animals there is a variety of diseases which may be mistaken for rabies, and undoubtedly often are. Experienced veterinarians, however, are correct in their diagnoses in the great majority of cases. Friedberger and Frohner mention a large number of conditions in the dog which may resemble rabies clinically.

It seems necessary to refer especially to the nervous form of dog distemper. In this disease there is sometimes a train of symptoms closely resembling those of certain cases of rabies. There may be the same convulsions and paralysis. The involvement of nasal and ocular mucosae, the intestinal symptoms, and eruption may be looked for in vain. However, a dog with distemper is said not to have the aggressive tendencies of a rabid one. An ugly dog with distemper would certainly *appear* more aggressive than a paralyzed and helpless rabid dog. There is some evidence that the resemblance between these two diseases extends further than the clinical features, and that their pathogenesis may be similar, although it certainly is not identical (see microscopic findings and inoculation tests). In the case of the dog the matter can be cleared up by microscopic examination and inoculation, when suitable post-mortem material can be obtained.

It is necessary to reiterate that the rabid dog is sick; is not necessarily running wild and furious; is frequently obedient up to a late stage; and often seems to have a "bone in his throat," or to have sustained injury to the back.

(4) *Termination.*—The great majority of persons and animals die of rabies after the first symptoms have developed. Therefore, if recovery occurs after the onset of the suspicious symptoms, the disease almost surely was not rabies. The statement that the disease is uniformly fatal does not, however, seem to be quite justified. Dogs are known to have recovered from rabies acquired under experimental conditions (Damman and Hasenkamp (25) and Högyes). There are not lacking reports of recovery in man; and, while the diagnosis is often found not to be justified beyond a doubt upon examination of the records, there still remains a probability in some cases that the disease was true rabies.

Josef Koch (52) adds new weight to the view that recovery from rabies is not impossible. He cites the varying and sometimes very long incubation period in animals experimentally inoculated as evidence that there are strains of virus of attenuated virulence from which recovery is possible, and gives instances of such recovery in dogs from *street virus inoculations* in his own experience. He cites four cases from Paltauf that died of intercurrent disease—alcoholism, apoplexy, and pulmonary embolism—after they had been bitten by rabid animals and whose brains conveyed rabies to inoculated animals, as indicating that the brain of a human being might actually be rabific without there being at the time any symptoms of the disease. Koch thinks, furthermore, that the cases of paralysis occurring during antirabic treatment are really cases of myelitis caused by the street virus infection.

No case must be regarded then as hopeless, and effort to effect a cure it not absolutely discouraged.

The duration of the disease is usually only a few days, and patients who survive a week or longer are probably suffering from another disease.

(5) *The post-mortem findings.*—As has been stated, these are for the most part inconstant and not absolutely characteristic. The absence of any other adequate cause of death should be determined. In the case of dogs, emaciation, foreign bodies in the alimentary tract, injection of the meninges and pharyngeal mucosa, and small hemorrhages in the central nervous system are extremely suggestive but not conclusive. It has long been hoped to determine a definite organism as the causal agent of the disease which by its demonstration in the tissues and isolation therefrom would render the diagnosis certain. This can not be said positively to have been accomplished, but the demonstration by Negri of certain bodies named after him has been a great advance in this direction.

Negri (74) has recently summed up his work upon the "parasite of rabies," *Neuroryctes hydrophobiæ* Calkins. He traces a developmental cycle for the organism, which he classifies with the Sporozoa. The bodies consist of a groundwork of protoplasm which contains "inner bodies." These latter are of two general types with transitional forms between them. The types are (1) small roundish and highly refracting; (2) larger, less refracting, roundish, or oval, or irregular. Both types are found in the same body. They are not artefacts, being found in unstained fresh tissues. In the smallest Negri bodies they are united and form a nuclear mass. As the parasite grows this nucleus increases in size and then becomes distributed throughout the protoplasm of the organism in the form of the smaller and larger inner bodies. These bodies then undergo a differentiation of their chromatin, which latter becomes separated and inclosed in a feebly staining capsule, constituting a "spore." The Negri body now consists of a mass of these spores. These are extremely minute—small enough to pass a bacterial filter. They may become dispersed throughout the nervous system, and when isolated would not be certainly differentiated from the granular structure of the latter. This explains why the parts of the brain which are very virulent may not contain Negri bodies in the cells. Golgi and Grassi confirm Negri's findings and concur in his interpretation of them.

These bodies are very generally admitted to be very constant in rabies and peculiar to it. Their availability for diagnosis is therefore assured in the majority of cases, but their exact significance is in dispute. The views of their significance are grouped as follows:

(a) *That they are the parasites causing rabies.*—This view is held by Negri himself and by a great many other observers. An able presentation of this attitude and very complete references to the literature on Negri bodies will be found in the articles of Williams and Lowden (119), and Williams. The supporters of this view urge the constancy (some 98 per cent) and specificity of the bodies to rabies, their morphology, which they consider that of the Protozoa, and the occurrence of various forms or stages which they regard as evidence of vital development.

(b) *That they are specific reactions of the invaded cells against the real parasites which are contained in the interior of the bodies and appear as granules differentiated by staining.*—This reaction is in the nature of an effort to isolate or sequestrate the offending microorganisms. The reason why they are found in greatest numbers in parts of the brain which are not especially virulent is that the cells of these parts are especially resistant and capable of exciting the limiting action. This view is held by Babes and his followers.

(c) *That they are cell degenerations or inclusions not connected necessarily with parasitic invasion.*—Achúcarro (1) claims to have shown that the glia cells of nerve centers undergo a degeneration in rabies which cause them to appear, when properly stained, identical with Negri bodies, and that these glia cells become incorporated into the larger cells and their processes and give rise to appearances indistinguishable from the Negri bodies.

Bearing upon the nature of these bodies are the following considerations:

Size.—It has been urged that they can not be the causal agent of rabies, as they are too large to pass the filters which fail to restrain the infective principle. This is hardly a valid objection, however, since it is not claimed that the visible forms are the only ones, but merely a stage of the development of the organism. Small plastic forms are assumed which are small enough to pass the filters.

Location.—The fact that they occur in great numbers in comparatively nonvirulent parts of the brain and are rare in the most virulent portions has been taken as evidence that they are not causative parasites; but Negri's explanation of this should be considered. If Stefanescu's (114) observation of these bodies in the parotid gland of a dog is confirmed in other cases, an important link will be added to the chain of evidence as to the parasitic nature of the bodies.

Morphology.—On morphological grounds alone they are regarded by many, including Calkins, as Protozoa, and stages of development are described; but others have claimed that their staining reactions are not those of typical Protozoa.

Standfuss (113) states that the mere finding of Negri bodies is not sufficient to establish the diagnosis. He found bodies almost indistinguishable from Negri bodies in "dog sickness."

Bodies somewhat similar to Negri bodies have been described in dog distemper and other conditions, but their differentiation is said by those who have described them not to be a difficult matter, except those described by Standfuss, already referred to.

Poor (92) has described such forms in a tetanic guinea pig; Lina Luzzani (62) in normal cats; Pace (86) in persons dying of old age, cerebral embolism, and aortic insufficiency; Babes (3) in a case of arsenic poisoning; Schiffmann (116) in Hühnerpest; Lentz (59) and Mazzei (71) in distemper; Pinzani (91) in a guinea pig dead of diphtheria toxin; Goffi (40) in the testicles of a healthy animal.

Before granting that indistinguishable or even similar forms may be found in distemper we must be sure that we are dealing with this disease. Text-book descriptions of distemper, and especially of the "nervous form," do not furnish us with any absolute criteria for the detection of the disease, which is alleged to be protean in its manifestations. It seems more reasonable to suppose that the bodies described came from cases of true rabies incorrectly diagnosed on clinical grounds as distemper, than to believe that bodies as definite in their structure and relations as the Negri bodies can be the result of more than one cause.

Marie apparently considers the Negri bodies as evidence of non-specific cellular reactions against certain microorganisms and toxins and other poisonous agents.

In expressing our own views regarding these bodies and their value for diagnostic purposes, it may be said that we have, so far, found them specific for the disease, and, when unmistakably present, diagnostic of rabies. They are not, however, always present or at least demonstrable in cases of rabies. When a person has been bitten by an animal having symptoms suspicious of rabies it is our custom to institute at once the preventive treatment, although we may not have been able to demonstrate the presence of the Negri bodies in the nervous system of the biting animal. Inoculation tests have shown this to be sound practice in a few cases where the Negri bodies were not found, since the inoculated animals have developed rabies. Again, the personal equation of the examiner must be taken into account. A person undertaking this work should be thoroughly familiar with the technic of demonstration and the appearance of these bodies before expressing an opinion as to their presence.

The custom of killing suspected animals immediately after they have bitten some one operates against the demonstration of Negri bodies, which may not be present in the early stage of the disease.

We believe that an experienced examiner may rely upon his diagnosis of rabies based on the presence of Negri bodies, but in their absence inoculation tests must be made before rabies can be excluded.

Among the changes, for which more or less diagnostic value has been claimed, must be mentioned the rabic tubercles of Babes, the ganglion changes of van Gehuchten and Nelis, the polynucleosis of Courmont and Lesieur, and the glycosuria of Rabieaux and Nicolas.

Babes, while admitting the diagnostic value of the Negri bodies, claims that the embryonal cell infiltration into the nerve tissue in the particular manner described by him is more constant and appears earlier. It is not, however, according to other observers confined to rabies. The same criticism applies to the lesions of van Gehuchten and Nelis, an embryonal cell infiltration of the connective tissue of the nerve ganglia, especially demonstrable in the plexiform ganglion of the vagus, with outgrowth of the endothelial lining of the ganglion cell capsules, and destruction of the large ganglion cells.

Courmont and Lesieur claim that in the dog there is always present a relative polynucleosis of the blood and lung juice. Whereas in this animal the polynuclears constitute normally 53 per cent of the leucocytes, in the rabid animal they form 90 per cent. In the absence of this condition they claim that rabies can be excluded.

These three microscopic lesions undoubtedly have some value from a diagnostic standpoint, and since they require no extraordinary technical methods for their demonstration they should be employed where time will permit; but they have been overshadowed of late by the search for Negri bodies which have largely occupied the attention of investigators.

Glycosuria as a symptom of rabies has been studied especially by Rabieaux and Nicolas. They examined the urine of a large number of animals, including dogs, cats, goats, and asses. They used the phenylhydrazin test and Fehling's solution, and identified the sugar by the polariscope. They concluded that while glycosuria is frequently encountered in the rabies of Carnivora, it is more pronounced, appears earlier, and is contant in herbivorous animals, constituting in the latter class a valuable aid to diagnosis. We have been unable to confirm their findings in the case of rabbits, in which they report finding sugar in all of 19 animals examined.

(6) *Inoculation tests upon animals.*—This remains the final resort in determining the diagnosis post-mortem. While the discovery of Negri bodies is regarded in many laboratories as conclusive evidence of rabies, rendering test inoculations superfluous, workers along these lines will do well to confirm their microscopic findings by ani-

mal inoculation until sure of their technic and satisfied of their ability to make a diagnosis from these findings. In the absence of Negri bodies test inoculation must be resorted to in all cases. For technique see page 63.

Irregularities may occur occasionally among inoculated animals, due to techical errors or relative immunity, but as a rule, the results will be at least as satisfactory as with the inoculation of any other pathogenic micro-organism.

Rabbits subdurally inoculated usually come down during the third week; guinea pigs often as early as the twelfth day or earlier. Sometimes, however, the period is much prolonged, as in a case mentioned by di Mattei, when the rabbits developed rabies after two hundred and seventy days, the delay being attributed to a loss of virulence dependent upon putrefaction of the material. We have observed an incubation period of one hundred and forty-six days in a rabbit inoculated in the muscles of the neck with putrefied material placed two days in glycerin. Many Negri bodies were found in the brain of this rabbit.

Schüder (112) gives the following figures relating to the incubation period in rabbits:

Method.	Virus.	First and second week.	Third week.	Fourth week.	Fifth week.	Sixth week.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Subdural	Street	10.5	83.2	5.0	1.0	0.3
Intramuscular	Phenolated	8.0	52.0	20.0	14.0	6.0

Inoculation with spinal fluid obtained during life is wholly unreliable, as it usually fails even in true cases of rabies.

The *Wassermann reaction*, deviation of complement, is said to have been obtained in a few experimental cases. It has not come into use up to the present time because of the uncertain state of the test and the difficulties attending its execution.

Heller and Tomarkin (47) show that the expressed juice of brains of rabid animals has in certain proportions when combined with antirabic serum (obtained from rabbits immunized with fixed virus) the power of deviating the complement so that hemolysis does not occur. The same, however, occurred when juice from normal rabbits was used. Normal rabbit *serum*, however, failed to restrain hemolysis in combination with normal or rabic material.

Baroni, Ciuca, and Ionescu-Mihaiesti (10) got completely negative results with the serum of rabbits subjected to Pasteur immunization, with the serum of a rabbit immunized by intravenous injections of fixed virus, and with Marie's sheep serum. They used as antigen a 10 per cent emulsion of fixed virus, as complement guinea-pig serum, and blood corpuscles from a dog.

Centanni (21) found that no reliance could be placed upon complement deviation tests with rabic antigen and antirabic serum, since the antigen alone had the power to fix the complement, as had also normal nerve tissue. The antirabic serum alone likewise fixed the complement in certain cases. The neurotoxic reaction takes precedence and obscures the specific reaction.

IMMUNITY.

The subject of immunity in rabies, as in many other infectious diseases, is one of great complexity, entailing the study of all the phenomena bearing upon the rationale of the disease, natural and experimental.

Natural immunity is enjoyed by certain of the lower vertebrata, the reptiles, for example; but in the amphibia (frogs) the immunity is not absolute, but relative, and is evidenced by an extremely long period of incubation and protracted course of the disease. Birds exhibit a less marked relative immunity, while there is not known to be a mammalian species that is immune.

Individual immunity does occur, however, in animals; and occasionally dogs and other animals are found which appear to be refractory to inoculation by the severest methods. The same, it would seem, might be reasonably predicated of human beings.

The occurrence of *hereditary immunity*, passive and transient in character and transmitted only through the immunized mother to her offspring, is indicated by the experiments of Remlinger (104). Konradi (54) states that this immunity extends only to the first generation, but endures for a long time.

Two important facts are known with regard to *acquired immunity:* First, that man and animals may be rendered immune by inoculations with the modified virus of rabies; second, that their blood acquires "rabicidal" properties, i. e., the power to render inert the virulent material exposed to its action *in vitro.* There are many other facts bearing on the modus of immunity, some of which have served only to introduce new problems, other to throw some light on the main question of how immunity is established.

Any conception of immunity in rabies necessitates a knowledge of the properties of rabies virus. The essential difference between street and fixed virus has already been pointed out (p. 20), but other changes of the virus in its course of modification from street to fixed virus remain to be considered.

Virus in its fixed condition, as indicated by its constant and irreducible incubation period, is in several ways different from street virus. It is generally considered to be *more virulent* for rabbits, inasmuch as it causes the disease so much more quickly. At the same time it has to some extent lost virulence for other animals higher in the scale, at least when injected subcutaneously. This is explained by perhaps a majority of observers upon the assumption of a toxin. i. e., a substance immediately poisonous and the product of the growth of the causative micro-organism, comparable to the toxins of *B. diphtheriæ* (a soluble toxin) or *B. pestis* (an insoluble or intracellular toxin). It is assumed that by the passage through rabbits the toxin element has been increased, hence the early onset and the paralytic symptoms; and at the same time the vegetative or reproductive power of the micro-organism has been increased (Marx). Its resistance to the inimical action of the body juices, however, is thought to be reduced, hence the innocuity upon subcutaneous injection. The toxins, according to this theory, are supposed to call forth the production of antibodies, which destroy the micro-organism and render its products inert before it has had opportunity, because of its lowered resistance, to injure the organism by its advancing growth.

This brings us to a consideration of the "toxin," which constitutes an important problem, since the demonstration of such a body has so far been insufficient for general acceptance. If no such substance exists, the present methods in general use of producing immunity in man may be advantageously modified, since it is really the fear of the effects of this hypothetical substance which at present prevents the general use of a more potent virus for immunization, which may confidently be expected to give a more rapid and intense immunity and be the means of reducing very materially the mortality after protective treatment.

The arguments advanced for and against the existence of a rabies toxin may be thus summarized:

IN FAVOR OF A TOXIN.	AGAINST A TOXIN.
The production of symptoms of fever, protracted emaciation, cachexia, and paralysis by virus filtered or otherwise treated so as to eliminate infection by a micro-organism.	That these symptoms are not typically rabiform and may be ascribed to other causes, notably the introduction of foreign nerve substance. Normal brain substance similarly treated has same action, but less marked. That these toxic symptoms are not constant. Heller and Bertarelli, 1904, observed none.
The occasional occurrence of paralytic symptoms apparently not due to rabic infection, occurring during or soon after antirabic inoculations.	Same as above, and that these symptoms are due to real street rabies, modified by the treatment to a mild or atypical course, or they are the manifestations of anaphylaxis due to the introduction of foreign proteid.
	That enormous amounts of fixed virus can be administered subcutaneously without toxic symptoms.

That the assumption of a toxin explains the difference between street and fixed rabies above described.	
That *immunity* to rabies may be conferred by increasing doses of filtered emulsions and those exposed to a high temperature.	No immunity in certain experiments.
Babes reports that by the use of filtration, alcohol precipitation, and dialysis a substance can be prepared which in a fresh condition and large doses induces in dogs, rabbits, and guinea pigs fever, hyperesthesia, paresis, marasmus, and death. The substance is complex and may well be an enzyme (Marx). Mechanically subdivided frozen virus is noninfective, but is toxic (Heller, Barratt).	That no specific toxic substance has been isolated in a pure condition, and that the products described may originate in the nerve tissue rather than in the micro-organism.
	That rabbit cord dried until it has lost infectious properties has lost also immunizing properties (Harvey and McKendrick).
	That large doses of fixed virus do not induce symptoms more quickly than small ones.
That some of the symptoms of rabies: fever, leucocytosis, hemorrhages, are those which we have come to recognize in other diseases as toxic (Babes).	That these symptoms are as well explained by the mechanical action of a micro-organism as by the action of a toxin (Harvey and McKendrick).
	That the conditions are in some respects parallel with those in smallpox where no toxin has been invoked to explain immunity, i. e., fixed virus: man-vaccine: man.
	That the specific activity of antirabic serum seems to be fully explained by its *antimicrobial* properties without assuming any antitoxic action.

Heller and Bertarelli (46) believe that the production of a poisonous substance does accompany the growth of the micro-organism in nerve tissue; but, like the toxin of tetanus, it is fixed by the nervous matter. Consequently it would play no dangerous part were fixed virus to be used for human immunization unless the combination were sufficiently unstable not to withstand certain unforeseen reactions (perhaps of a neurolytic nature) within the economy.

Högyes (49) explains artificial immunity conferred by the injection of fixed virus as follows:

It appears that the action of successive injections of dilutions of fixed virus, increasing from weak to strong, or of the emulsions of dried cord, is primarily this: that the rabies toxin contained in these arrives earlier at the cells of the central nervous system by way of the blood and lymph circulations than does the slowly progressing growth of the rabies microbe along the nerves. So that upon their arrival at the nerve centers the protoplasm of the nerve cells has already become accustomed to the action of rabies toxin and consequently the virus, introduced during the preventive inoculations or introduced for purposes of (experimental) infection, can no longer affect the nerves and cause the above-described chromatolytic changes in them. "The microbe finds only toxin-proof elements."

Marie calls attention to the fact that in immunization with dried cords a relatively high proportion of toxin to living micro-organisms may be expected in the earlier doses administered, since the drying at 22° can have had little influence upon the toxin, while it is known to gradually reduce the infectious properties of the cord, presumably by a numerical decrease of the micro-organisms. Applying to this supposition, however, the idea of fixation of liberated toxin by the nerve tissue, would it not be reasonable to suppose that the toxin contained in the dead parasites would be fixed by the surrounding nerve elements in the virus itself and thus rendered innocuous to the cells of the person or animal to be immunized?

To return to the fixed virus: Whether or not this contains a dangerous toxin, it is the fear of such a substance that has to a great extent deterred Pasteur institutes from using fresh (unattenuated) fixed virus for the production of immunity, inasmuch as it is now widely accepted that there is little to fear in the way of rabic *infection* from the subcutaneous exhibition of this substance. This belief is supported not only by actual experiment which certain observers have made upon their own persons, but by the apparent success of a method which is actually based upon the use of fresh fixed virus, namely, Ferran's method (q. v. under treatment). Ferran has stated that there is a toxin which excites the production of antibodies which destroy the virus. Hence, he claims large doses are less likely to cause *infection* than small ones, since in the latter there is proportionately little of this antibody-evoking substance, while the living virus capable of growth is just as potential of producing the disease as it would be in larger doses.

While Ferran's results are reported to be good, it was not so with one of his would-be followers, Bareggi (9), who, in an earnest effort to improve upon the Pasteur method as generally employed, adopted that of Ferran, *as he understood it*, with the result that five of his patients died within ten days of each other, and that in consequence his institute was closed by the Italian Government. The symptoms of these persons were those of the paralytic form, and their brain material, when inoculated into animals, produced rabies in the normal period for fixed virus. This, then, constituted an infection, and not an intoxication. These cases have undoubtedly dampened the ardor of many who have wished to improve upon the usual Pasteur treatment in the direction of intensification; but it should be remembered that they are unique and counterbalanced by thousands of cases in which fresh fixed virus has been used with impunity. They are probably explained by the fact that *fixed virus, so called, is not a substance of uniform virulence.* It is true that a given strain of virus comes by repeated passage to a state beyond which there is no apparent change, and that all strains of virus thus treated arrive apparently about the same stage when finally "fixed." But these strains do differ among themselves considerably.

If we examine a large number of original descriptions of the properties of fixed virus we shall find that one author will say that in rabbits inoculated subdurally with fixed virus the animals show decided symptoms on the sixth day; another will say after six days or on the seventh; another, on the fifth day (Ivo Novi) (83); not a great difference, to be sure. But in describing the duration of the disease a greater difference is apparent, death being said to occur all the way from the sixth to the twelfth day.

In a verbal communication from a physician formerly in charge of the Pasteur treatment in Mexico, it was learned that the strain of fixed virus employed there induces symptoms in rabbits in three or four days, and deaths on the sixth day. This virus came originally from a rabbit inoculated personally by Pasteur. When originally received, some sixteen years ago, its incubation period was eight days. This steadily decreased for seven or eight years, since which time it has been fixed at the present degree of virulence.

Marie states that the virus of Santiago, Chile, kills rabbits regularly in six or seven days.

Passage of virus in animals other than the rabbit.—It is generally stated on early authority that serial experimental passage of virus in *dogs* results in diminution and final loss of virulence. If this were true the disease would disappear spontaneously from among these animals unless it were reenforced from time to time by passage through some susceptible species. The experiments of Marie (65) have shown, however, that the virus does not lose it potency when passed serially through dogs, but, on the contrary, tends to become fixed with an incubation period, when transferred to rabbits, of eight or nine days.

In rats, street virus becomes rapidly exalted in virulence, even with a few passages.

In foxes, cats, and the herbivivores also an augmentation of virulence is said to occur.

An *attenuation* and *final loss* of virulence is observed when monkeys, frogs, and birds are serially inoculated. In fact, the monkey was utilized by Pasteur to attenuate the virus in the first attempts to obtain a noninfectious vaccinal material.

Location of the virus within the body.—The central nervous system and peripheral nerves harbor the virus with great constancy, and it was the appreciation of this fact that led to the opening up by Pasteur of the whole field of modern research on rabies.

The infectiousness is variable in different parts of the nervous system, the peripheral nerves being less virulent than the centers.

Nitsch (79) found that 0.1 mg. of the cortex of the brain (fixed virus) was lethal for rabbits in seven to nine days, while 0.5 mg. from the middle of the cord was not so, but 1.0 mg. was lethal. The medulla he found to be some five times more virulent than the middle of the cord. Later he (81) states that the brain is almost one hundred times as virulent as the cord. He claims that the gray matter is much more virulent than the white during life, but that this difference diminishes after death, due, it is assumed, to a post-mortem diffusion of the micro-organism. The cortex is somewhat more virulent than the cornu ammonis and corpora quadrigemina, which are equivalent. The sympathetic ganglia are stated to be very little virulent and the retina not at all so.

There is a widespread conviction that after death a diffusion of the virus takes place so that organs which during life would not contain it are found virulent after removal at autopsy.

The *cerebrospinal fluid* has proved avirulent in the great majority of cases where it has been investigated.

The *skin* may possibly contain the virus at the point of inoculation, the evidence of this resting upon the experiments of Pace (85), who transmitted rabies to rabbits by inoculation with the cicatrix from a fatal case of human rabies.

Certain glands contain the virus with considerable constancy if certain animal species be considered. The *salivary glands* of dogs are very constantly infectious, much less often those of rabbits, and very rarely those of man. Roux and Nocard (110) found that the saliva of a dog was virulent some three days before the onset of definite symptoms, and Rabieaux (94) claimed that the submaxillary gland could become virulent before the brain and cord. This is against the accepted idea that the virus proceeds from the brain to the salivary glands by way of the nerves.

According to Remlinger (101) the saliva of a dog may remain virulent five days after recovery from rabies, which sometimes although rarely happens. This animal had been inoculated with fixed virus.

In *man* the salivary glands are, it appears, seldom invaded by the virus except perhaps by a post-mortem diffusion of the virus. The positive results obtained by Bardach have not been obtained by later observers in the majority of cases. However, successful inoculations with the saliva of persons suffering from rabies are reported by Bertarelli (12), and by Nicolle and Chaltiel (78), and also Magendie's original transmission of rabies from man to dogs was by means of saliva. Bertarelli, in his last-mentioned work, seems to have shown that the virus is conveyed to the salivary glands by way of the nerves.

Aqueous humor of the eye.—This fluid is generally considered avirulent, but positive results from its inoculation are reported by Courmont and Nicolas (24).

Blood.—If the virus could be demonstrated in the blood stream, it would be reasonable to expect the same result in any blood-containing organ. Results of the experiments with blood of persons and animals suffering from rabies have, however, been for the most part negative. Marie (64) was able to demonstrate virulence of the blood serum of animals artificially inoculated even before their symptoms had developed. He makes no claim that this is the general rule.

Joseph Koch states that he also has demonstrated the infectivity of the blood.

We consider it extremely likely that the period at which the blood is drawn for examination has much to do with the presence or absence of infectious properties and that the same is true of other tissues.

In this connection must be considered the question as to whether the blood stream may convey the virus to the fœtus. Evidence of such conveyance would be gained by the development of rabies in the new born if other possible means of infection, such as nursing, licking of the offspring by the mother animal, etc., could be excluded. So far as we know, no placental transmission of rabies in the human species has been published, but several instances of it have been observed in animals under experimental conditions. (Loir (61), Konradi (53), Perroncito and Carità (89), and recently Damman and Hasenkamp (25).)

Negative results of experiments along the same lines are recorded by Bombicci, Zagari, and Repetto.

There are a number of clinical observations of placental transmission in the lower animals. (Bollinger (14) cites Callicac's case; Friedberger and Frohner (35).)

Milk.—As in the blood, the virus seems to be found in the milk of rabid animals with great infrequency, but there are sufficient instances on record of the virulence of milk to cause us to regard this fluid as dangerous when taken from a cow suffering from rabies.

Bardach (8) records the case of a woman suffering from hydrophobia whose milk was demonstrated to contain the virus by inoculation of animals. A child which she had been nursing remained well.

Nocard also obtained positive results with milk from a rabid bitch. Nicolas (76) had only negative results from his experiments with herbivores.

The danger would, of course, be greater through contamination of abraded surfaces upon the hands of the milker than through ingestion of the milk.

The *liver*, *spleen*, and *spermatic fluid* appear never to harbor the virus. The *pancreas*, *lachrymal glands*, and *suprarenal capsules*, however, seem to be virulent in a large number of instances.

Resistance of the virus to physical and chemical agents.—Grinding: Experiments with virus subjected to grinding in a frozen condition (MacFadyean's apparatus) have shown that *infectiousness* may be destroyed by mechanical subdivision. The ground substance, however, had toxic properties.

Pressure: Heller (45) has shown that rabic virus resists a pressure of 200 to 350 atmospheres (Buchner press).

Centrifugalization: This is competent to render the supernatant fluid of an emulsion avirulent. The sediment, of course, is virulent.

Heat: Variations in the results of the action of heat upon rabies virus obtain just as in the determination of the thermal death point of bacteria. Marie quotes Celli to the effect that the virus is destroyed in one hour at 60° C.; Roux, in a few minutes at 60°; Högyes, in thirty minutes at 52° to 58°. Marie found that a centesimal emulsion was rendered avirulent by exposure to 60° for thirty minutes.

The following figures indicate the results of heating, as observed by Babes and by Puscariu (from Marx (69)):

Minutes.	Degrees.	Cord kills rabbits in—
		Days.
40	58	(*a*)
32	58	20
24	58	16
24	56	16
16	58	12
8	58	12
4	58	11
2	58	9

[a] Not killed.

Puscariu says that heating ten minutes 80°, 70°, and 60°, the animals live; 50°, animals die after eleven to twelve days; 45°, animals die after eleven days; 40°, animals die after ten days; 35°, animals die after nine days; 30°, animals die after nine days; unheated, animals die after eight days.

Cold.—Barratt subjected rabies virus to the temperature of liquid air (—190° C.) for three months without destroying its virulence.

Desiccation.—At 23° C., the virulence is usually lost in five or six days; at lower temperatures, the loss is more slowly effected; at higher temperatures, more rapidly, for example, at 35° C., *in vacuo*, the virulence is retained for 121 hours; in carbonic acid gas, 198 hours; in air, only 56 hours. Rapid dessication, however, under certain conditions is not destructive of the virulence, since Vansteenberghe (117) found that a rabic brain, reduced to a pulp and spread in a very thin layer, *in vacuo*, and rapidly desiccated for twenty-four hours in the presence of sulphuric acid, furnished a product which was capable of causing the disease in rabbits, with the same incubation period that was required for the same virus in the fresh state. Rabic virus prepared in this way is not susceptible of attenuation under the conditions usually obtaining in laboratories for the attenuation of virulent cords, even at the end of several months; in the form of powder this rapidly desiccated virus, preserved in stoppered tubes in the dark for nine months, was still capable of causing rabies in rabbits on the seventh day when subdurally inoculated. These findings were confirmed by Remlinger and Nouri (105) and Marie. The former state that it is immaterial whether the desiccation is effected in the presence of H_2SO_4, KOH, or atmospheric air; they seem to think that the rapidity of desiccation is dependent rather upon the thinness of the layer of brain pulp exposed to the drying action. In their experience, under the most favorable conditions, the powdered virus did not retain its virulence longer than three months. It is not applicable for treatment by the method of Högyes.

Harvey and McKendrick (43) claim that the loss of virulence is nearly proportionate to the loss of water extracted through drying.

Light.—This is said by Celli to destroy the virulence in forty hours (i. e., sunlight).

Rontgen rays.—These appear to have no significant action upon the virus, according to the experience of Högyes, Frantzius, Calabrese, and Ceni.

Radium.—It is claimed by Tizzoni and Bongiovanni that radium exerts a rabicidal action upon the virus *in vitro* and a curative action upon animals even after the onset of the symptoms. These claims have not been substantiated by other observers, and are contested by Calabrese, Rhens, J. Danysz, and Ivo Novi.

Dilution.—It is possible to dilute emulsions of rabies virus until a small amount will no longer transmit the disease when inoculated subdurally in rabbits. According to Högyes, this degree is attained with a dilution of 1:10,000. Nitsch (79) found that larger amounts (1 to 3 c. c.) of this dilution nearly always caused rabies.

Filtration.—Emulsified rabies virus is stated to pass the pores of certain Berkefeld filters, but not the finer Chamberland filters. Bertarelli and Volpino claim that fixed virus passes finer pores than street virus, and direct attention to the corresponding size of the Negri bodies in these two forms of the virus. Experiments upon the filterability of virulent suspensions must be made and interpreted with caution. Imperfections of the filter itself are difficult to exclude. At the Hygienic Laboratory we have never succeeded in obtaining a virulent filtrate of fixed virus emulsions, using a Berkefeld candle and the ordinary water-pump vacuum. Marie states that no bodies can be discovered in the filtrate by the new ultramicroscopic methods.

Chemical agents.—Rabies virus is sensitive to the action of acids and alkalies, but is more resistant to chemical disinfectants than are bacterial emulsions. The difficulty in estimating the susceptibility of virus to chemical agents is that we can not note the effect of these substances upon the growth of the organism in a test tube as in the case of bacteria which may be cultivated, but must introduce mixtures containing the chemical into the body of an animal, and, to be sure of results, under the cerebral dura. Results are thus vitiated by the action of the chemical upon the animal. Fermi (32) has sought to avoid this objection by injecting the mixtures subcutaneously into rats, which are very susceptible to the virus which he employs in this manner. Until his results shall have been more fully tried out it is deemed inadvisable to accept as accurate any data as to the rabicidal action of chemical substances.

The resistance of rabies virus to biological influences.—Putrefaction of rabic nerve substance may proceed to the extent of liquefaction without completely destroying the virus. There is reason to believe, however, that the virulence may be reduced by putrefaction so that the incubation period in inoculated animals will be much prolonged.

When rabies virus is introduced into a vertebrate host it meets with conditions inimical to its growth which vary in intensity according to the site of inoculation, the species, and the amount and character of the virus. Thus, virus injected into the lymph sac of the frog is said to lose its virulence in a short time. The peritoneum exerts an unfavorable action on the virus, and attempts to inoculate animals by this route are generally unsuccessful (Remlinger (99)).

The gastric juice is so destructive of the virus that infection would hardly seem possible were it not that Fermi (33) and Remlinger (102) have succeeded in causing the disease in rats by this means.

When we consider the subcutaneous injection of virus we must distinguish the comparatively greater resistance of street virus from that of fixed virus. Apparently there are agencies at work in the subcutaneous tissues which are capable of destroying the virus under certain conditions. In the work of Kraus, Eisler, and Fukuhara (55), leucocytes or cells of organs were capable of, as they say, *adsorbing* the virus of rabies from an emulsion *in vitro* if digested with it at 37°. Carbon and certain acids and alkaline substances possessed the same properties. All these substances had a similar action on the vaccines of Hühnerpest and cowpox.

This furnishes a hint that the formed elements in the subcutaneous tissues cooperate with the serum in the destruction of virus introduced under the skin. Since leucocytosis is known to occur during immunization with fixed virus (Nicolas and Bancel (77)), there is an abundant supply of formed elements ready to exert whatever inimical action they are capable of upon the newly introduced virus. This leucocytosis is caused also by the introduction of normal nervous substance, so that whatever fraction of the total immunity is to be attributed to leucocytic action is due probably to the nervous tissue *per se* and not to the specific elements of the virus.

A consideration of these properties leaves no doubt that the virus contains a living morphological entity which is the cause of rabies.

Having described in brief some of the properties of rabies virus it is necessary to discuss the properties of the serum which are induced by immunization with this substance. The most striking property not possessed by normal serum is that of being able to destroy the activity of the virus of rabies *in vitro* by contact with it. The blood serum of a person or other mammal subjected to a course of injections by Pasteur's method, when mixed in certain proportions with an emulsion of fixed or street virus, will cause the virus to become inert when injected subdurally into a test animal. This property is not possessed by the serum of animals suffering from rabies. Some time is necessary for the development of this property. Kraus and Heller were unable to detect it until twenty-two days after the termination of Pasteur treatment in five persons examined. Its duration is, however, of considerable length, since the same observers were able to demonstrate it in the same patients eighty-five days later. In a patient whose serum was examined at the Hygienic Laboratory two years after treatment at a Pasteur Institute very little antirabic activity was shown. All the animals injected with the virus-serum mixtures died of rabies, but there was a relative retardation of the time of onset and death corresponding to the amount of serum used.

According to Marie (68) the action of the serum upon the virus appears to be of the nature of a fixation of some component upon some specific body in the virus. Serum "saturated" with a portion of virus is deprived of the property of destroying the virulence of another portion after its recovery from the mixture by centrifugation. Normal nerve tissue, however, does not remove the antirabic property. The union between the hypothetical antibodies and antigen is instable, since virus so neutralized may regain its virulence after washing with salt solution. There is no evidence that antirabic serum posseses a neurotoxic action, which might account for its antirabic properties.

An interesting fact in connection with the study of antirabic serum is that its activity is sometimes confined within narrow limits, and this is especially true in case the treatment of the animal producing the serum has been of long duration. To illustrate: A serum active against a definite emulsion of virus—say 1 per cent—when added in the amount of 1 c. c. might be inactive in the amounts of 0.1 and 5 c. c. The explanation given of this phenomenon is that the smaller amount was insufficient to neutralize all the virulent units, while the larger amount (5 c. c.) was hampered in its action by the excess of immune bodies over the complement which is necessary to effect the destruction of the microörganism through their mediation. Marie has noted an increase of potency of serum subjected to a temperature of 56° C.

While the serum of normal mammals has been found inactive against rabies virus by nearly all observers, the serum of some especially resistant nonmammalian species has been shown to be active when added to fixed virus in large amounts. Marie found the serum of fowls to be active in the proportion of 3 parts of serum to 1 part of fixed virus (1:100).

From immunized sheep a serum having a titre of 1:40 F. V. $_{\frac{1}{100}}$ has been obtained.

Notwithstanding the marked antirabic properties which may be demonstrated in a serum *in vitro*, its action within the body experimentally has not given us reason to expect much usefulness in a therapeutic way. This apparently depends upon the following facts: (1) That the action of the serum is probably purely microbicidal; hence, if a toxin plays a part in the pathology of rabies no antagonism to this substance could be expected from the serum; and again, in developed rabies where the nerve cells have already been destroyed, a mere killing off of the causative organism would not result in *restitutio ad integrum*. (2) That the immunity conferred during the period of incubation is, like all passive immunity, transient in character. The microörganism might be temporarily held in check by an injection of serum; but after the rapid disappearance of the immune properties of the serum it is reasonable to suppose that it would proliferate again and produce the disease. Animal experimentation has borne out this supposition to some extent, since in the majority of tests a prolongation of the incubation period only has resulted from the injection of antirabic serum. The wonderful results reported by Tizzoni and his coworkers have unfortunately not been reduplicated by other investigators.

Literature.—Most of the important articles on serum therapy are referred to in Marie's "Etude experimentale de la rage." Paris, 1909. We would refer also to the articles by Poor and Friedmann, Collected Studies from the Research Laboratory, Department of Health, City of New York, volume 2, and Semple, in the Lancet, 1908.

In summing up the data on the modus of active immunization against rabies virus the following facts seem to be established:

1. That rabicidal properties appear in the blood serum.

2. That the brain cells in high degrees of immunity resist the action of virus directly applied to them.

3. That there is evidence that leucocytosis is induced and that leucocytes have the property of at least temporarily rendering the virus inert.

On these data we advance the hypothesis that the first step in the establishment of immunity is the production of leucocytosis. That the next is the production of antibodies in the blood serum, and that finally the nerve cells themselves may in some cases acquire resistance to the invasion or at least to the pathogenic action of the microörganism.

We do not know the relations of these factors one with another. They must be to a certain extent independent, at least as regards serum and cellular immunity, since animals which after treatment may furnish a serum of high antirabic potency, may nevertheless at the same time be susceptible to subdural infection.

TREATMENT OF RABIES.

A. *Of the developed disease.*—Up to the present time no cure for the developed disease has been discovered. From time immemorial there have been advocated various drugs and procedures for which their discoverers have claimed curative properties, but none have stood the test of time. Nearly all the drugs of the pharmacopœias have been used at one time or another in this malady, besides a great number of weird and noxious combinations. Cures are reported from the use of diaphoretic drugs and measures, especially the Russian bath, but the diagnosis in many of these cases rests upon very insecure foundation. However, it must in fairness be admitted that it is possible or even probable that recovery has taken place in man, since it is well known to have occurred in dogs; but to what extent this result has been due to treatment remains undetermined.

In recent years we have come to look to serum therapy for the cure of acute infectious diseases, but the results in the cases of diphtheria and cerebro-spinal meningitis obtained with immune sera, brilliant as they are, are no better than those obtained in malaria and sleeping sickness, for example, with quinine and the arsenical preparations. The former are bacterial infections; the latter, protozoan. What rabies is, is undetermined, but the weight of evidence seems to favor its inclusion among the latter. Hence, from our experience in the treatment of other infections, we may as well expect the specific for rabies to be a mineral or a vegetable drug as a serum or other substance specifically related in its origin to the ultimate cause of the disease.

Antirabic serum alone has not so far saved a human being after the development of rabies, although it has undoubted prophylactic properties with definite limitations and is capable of destroying rabies virus *in vitro*.

For the present we must treat developed rabies in man symptomatically. Chloroform inhalations, prolonged if necessary, for the control of painful spasm; chloral and the bromides *per rectum*, or, possibly, curare subcutaneously. Morphine is recommended in large doses by some authors, but this may increase the nervous excitement and mental suffering. Hypnotic drugs are usually, even in very large doses, without appreciable effect. Where the patient can not swallow food, rectal alimentation is probably to be preferred to feeding by the stomach tube, the introduction of which is painful, difficult, or impossible.

Intubation or tracheotomy is probably useless for the relief of dyspnœa and suffocation, as there is generalized spasm of the respiratory muscles and the glottis may be patent all the time.

Mechanical restraint is generally unnecessary and should not be resorted to except in the violent maniacal forms. The sick room should be quiet, warm, and free from drafts. Admission to it should be as limited as possible. Attendants should preserve a calm and cheerful demeanor, and should only speak of the patient's disease to reassure him. Undoubtedly the suffering is often increased by nervous excitement and by the relation by an indiscreet attendant in the patient's hearing of dismal tales of suffering in similar cases.

B. *Prophylactic treatment of exposed persons—Local treatment of the wound.*—Cauterization has been advocated and practiced since the time of Celsus, and probably longer, since he undoubtedly took his descriptions of the process from an earlier source. When properly carried out it is undoubtedly of some benefit. It has been shown by experiment to prolong the incubation period, even when it did not prevent the extension of the infection; and in this way would allow more time for the establishment of immunity by the antirabic inoculations. It should be practiced as soon after the injuries as possible, but may be employed at any time while the wound remains open.

Actual cautery is efficient only if immediate and thorough. It involves too much suffering and destruction of tissue to be of general use. Another objection to the actual cautery is that it is difficult to reach all depths of the wound and that unreached pockets may exist where the virus is not destroyed.

The best method is to thoroughly touch all parts of the wound down to its depths with nitric acid. Carbolic acid is probably less effective. Silver nitrate is said to be very inefficient, and its use is not advised. Aside from cauterization, this wound is to be treated along the general principles of surgery. Promising experimental results with Bier's method have recently been reported by Cano (19).

Cauterization of wounds does not in the least supplant the antirabic inoculations.

General treatment.—The patient should endeavor to keep himself in good general health. Nervous excitement, fatigue, and all forms of excess, especially alcoholic, are to be avoided. Cold bathing and exposure to wet and cold are unfavorable.

Preventive inoculations.—The Pasteur treatment for the prevention of rabies in exposed persons is designed to confer immunity during the period of incubation. The production of this immunity, as at present practiced, is a long process, but fortunately the period of incubation in the majority of cases is longer. In persons in whom from a combination of the factors already mentioned (p. 21) the incubation period would be very short the Pasteur treatment fails. It also fails in certain individuals who can not be immunized, from some unknown peculiarity of their constitutional make-up. This condition is paralleled in other diseases. To this extent the present method of preventive inoculation is not ideal. It can undoubtedly be improved, but just how to do this with safety is a problem which has long been before us.

The principle upon which the Pasteur treatment is based is the production of immunity by the inoculation of rabies virus so modified as to render it innocuous. Pasteur first accomplished this by serial inoculation of monkeys, in which animal the virus loses its potency. Test animals inoculated with the spinal cord of these monkeys become immune to subsequent infection with virulent rabies virus. This method was, however, obviously inapplicable to man on a large scale. He finally devised the method at present in use, which involves the treatment of the virus in two steps: First, the serial passage of the virus in rabbits until a fixed degree of virulence is reached; second, the attenuation of this rabbit virus by desiccation.

The first of these processes is, in the light of our present knowledge, the more important, since the fixed virus is so modified as to be relatively innocuous to man. The second of these processes has been modified in various ways, or even dispensed with.

The modifications proposed or in use will be briefly indicated:

(1) *The use of unmodified fixed virus* was advocated and introduced by Ferran, of Barcelona. The results are stated to be good, but the selection of patients for treatment is stated by Remlinger to be favorable to a low mortality, inasmuch as no patient is admitted to treatment who has been bitten as long as ten days before application. Ferran emulsifies 0.08 gm. of the cord of a rabbit dead of fixed virus infection, with the aid of sand to comminute it thoroughly and to produce a fine emulsion, using 8 c. c. of fluid, apparently salt solution or bouillon. Six c. c. of the top fluid are immediately injected subcutaneously into three different portions of the body, 2 c. c. in each. These injections are repeated on five successive days. In severe cases the treatment is repeated after an interval of five to ten days (Frosch (36)).

A similar treatment has recently been employed by Proescher (93), in Pittsburg. He states that he uses does fifty times as great as those of Ferran, and has had no death from rabies in forty persons treated. He first demonstrated the innocuousness of his particular strain of fixed virus, which he designates "Pittsburg," by injecting a whole brain of a rabbit, dead of its infection, into each of two persons intramuscularly, no ill effects following. He had reduced the number of injections from two daily for ten days to one daily for six days, and contemplated a further reduction.

(2) *The dilution of fresh fixed virus.*—This method was devised by Högyes. He claims that in Pasteur's method the so-called attenuation was in reality but a numerical diminution of the rabies organisms present, a view entertained by Pasteur, and that this could be more accurately controlled by simply diluting the fresh virus with salt solution, increasing the dosage as the treatment progressed by increasing the strength of the emulsion. Högyes claims that more accurate dosage is possible by this method, and that the method employed experimentally on dogs gave more favorable results. An advantage pointed out by Harvey and McKendrick is that smaller amounts of nerve tissue in proportion to the amount of virus *per se* are inoculated, inasmuch as a large proportion of the nerve tissue of dried cords is inert as regards its power to immunize. These authors ascribe the paralytic symptoms which sometimes occur during or after immunization with dried cords to anaphylaxis induced by the repeated injection of this foreign nerve substance, and state that these symptoms are not observed with the dilution method of immunization.

The method is briefly as follows: An original emulsion of fixed virus is prepared from the spinal cord of a rabbit killed or dead of fixed virus infection, by rubbing it up with sterile salt solution (0.7 per cent) in the proportions of 1 to 100. By appropriate additions dilutions are then prepared varying from 1:200 to 1:10,000. These dilutions are now to be used for immunizing according to the following formulæ, injecting them subcutaneously:

Simple cases: Slight hand or foot wounds.

Day.	Time.	Dilution.	Cubic centimeter.	Day.	Time.	Dilution.	Cubic centimeter.
First	a. m.	1:10,000 &	3	Eighth	a. m.	1:1,000	1.5
		1:8,000	3		p. m.	1:500	1
	p. m.	1:6,000 &	3				
		1:5,000	3	Ninth	a. m.	1:200	1
Second	a. m.	1:5,000	3	Tenth	a. m.	1:6,000 &	3
	p. m.	1:2,000	2			1:5,000	3
					p. m.	1:2,000	2
Third	a. m.	1:2,000	2				
	p. m.	1:1,000	1.5	Eleventh	a. m.	1:2,000	2
					p. m.	1:1,000	1.5
Fourth	a. m.	1:1,000	1				
	p. m.	1:500	1	Twelfth	a. m.	1:1,000	1.5
					p. m.	1:500	1
Fifth	a. m.	1:200	1				
				Thirteenth	a. m.	1:200	1
Sixth	a. m.	1:6,000 &	3				
		1:5,000	3	Fourteenth	a. m.	1:100	1
	p. m.	1:2,000	2				
Seventh	a. m.	1:2,000	2				
	p. m.	1:1,000	1.5				

Severe cases: Head and face wounds.

Day.	Time.	Dilution.	Cubic centimeter.	Day.	Time.	Dilution.	Cubic centimeter.
First	a. m.	1:10,000 &	3	Tenth	a. m.	1:1,000	1.5
		1:8,000 &	3		p. m.	1:500	1
		1:6,000	3				
	p. m.	1:5,000 &	3	Eleventh	a. m.	1:200	1
		1:2,000	2				
				Twelfth	a. m.	1:6,000 &	3
Second	a. m.	1:5,000 &	3			1:5,000	3
		1:2,000	2		p. m.	1:2,000 &	2
	p. m.	1:1,000 &	1.5			1:1,000	1.5
		1:500	1				
				Thirteenth	a. m.	1:1,000 &	1.5
Third	a. m.	1:200	1			1:500	1
					p. m.	1:200	1
Fourth	a. m.	1:6,000 &	3				
		1:5,000	3	Fourteenth	a. m.	1:6,000 &	3
	p. m.	1:2,000 &	2			1:5,000	3
		1:1,000	1.5		p. m.	1:2,000 &	2
						1:1,000	1.5
Fifth	a. m.	1:1,000 &	1.5				
		1:500	1	Fifteenth	a. m.	1:1,000	1
	p. m.	1:200	1		p. m.	1:500	1
Sixth	a. m.	1:6,000 &	3	Sixteenth	a. m.	1:200	1
		1:5,000	3				
	p. m.	1:2,000 &	2	Seventeenth	a. m.	1:6,000 &	3
		1:1,000	1.5			1:5,000	3
					p. m.	1:2,000 &	2
Seventh	a. m.	1:1,000	1.5			1:1,000	1.5
	p. m.	1:500	1				
				Eighteenth	a. m.	1:1,000	1.5
Eighth	a. m.	1:200	1		p. m.	1:500	1
Ninth	a. m.	1:6,000 &	3	Nineteenth	a. m.	1:200	1
		1:5,000	3				
	p. m.	1:2,000 &	2	Twentieth	a. m.	1:100	1
		1:1,000	1.5				

Between these formulæ gradations may be arranged to suit individual cases.

This method has been extensively employed with good results at Budapest, and the same principle has been adopted in India.

(3) *Fixed virus attenuated by drying.*—The original method of Pasteur, the one most extensively practiced, has the advantage that it may be administered by private physicians at a distance from the laboratory, since the dried virus can be preserved for some time by glycerinization and shipped in this condition from the laboratory. Again, its wide application has created a confidence in its use which the less extensive experience with other methods, however worthy they may be, has not begotten.

The original schema of its employment has been modified from time to time, so that scarcely two institutions now follow identical procedures. This modification has been effected along three lines: (1) With regard to the time consumed by the treatment, either lengthening or shortening the original period of fifteen days; (2) dispensing with some of the more attenuated cords; (3) increasing or diminishing the dosage given at individual injections. In addition must be mentioned the unintentional modifications caused by the use of small rabbits, the relatively smaller cords of which desiccate, and consequently attenuate more rapidly than do those of rabbits of standard weight (2,000 grams); and by drying cords at temperatures other than 23° C. At lower temperatures the cords attenuate in virulence less rapidly.

The schemata devised by Pasteur and until recently followed at the Institute at Paris are three, the least intense of which is rarely employed. The other two, together with modified schemata advocated by other authorities, are inserted here to illustrate the direction and extent of the modifications:

Pasteur's original schemata (Marx).

Light schema.			Intense schema.		
Day of treatment.	Age of dried cord.	Amount of injected emulsion.	Day of treatment.	Age of dried cord.	Amount of injected emulsion.
	Days.	*c. c.*		*Days.*	*c. c.*
First	14 13	3 3	First	14 13 12 11	3 3 3 3
Second	12 11	3 3	Second	10 9 8 7	3 3 3 3
Third	10 9	3 3	Third	6 6	2 2
Fourth	8 7	3 3	Fourth	5	2
Fifth	6 6	2 2	Fifth	5	2
Sixth	5	2	Sixth	4	2
Seventh	5	2	Seventh	3	1
Eighth	4	2	Eighth	4	2
Ninth	3	1	Ninth	3	1
Tenth	5	2	Tenth	5	2
Eleventh	5	2	Eleventh	5	2
Twelfth	4	2	Twelfth	4	2
Thirteenth	4	2	Thirteenth	4	2
Fourteenth	3	2	Fourteenth	3	2
Fifteenth	3	2	Fifteenth	3	2
Sixteenth	5	2	Sixteenth	5	2
Seventeenth	4	2	Seventeenth	4	2
Eighteenth	3	2	Eighteenth	3	2
			Nineteenth	5	2
			Twentieth	4	2
			Twenty-first	3	2

According to this schema, head injuries require twenty-one days of treatment.

Babes's modification for a man who applied for treatment six days after having received a finger bite.

Day of treatment.	Age of dried cord.	Amount of injected emulsion.
	Days.	*c. c.*
First	12 11 12 10 10	4 4 4 4 4
Second	9 9 8 8	4 4 4 4
Third	7 7 6 6	4 4 4 4
Fourth	5 5 4 4	4 3 3 2
Fifth	3 3 2	2 2 2
Sixth	Pause.	....
Seventh	7 6	4 4
Eighth	5 4	3 3
Ninth	3 2	2 2
Tenth	2 1	2 2
Eleventh	Pause.	....
Twelfth	8 7	2 2
Thirteenth	7 6	2 2
Fourteenth	6 5	2 2
Fifteenth	5 4	2 2
Sixteenth	4 3	2 2
Seventeenth	3 2	2 2
Eighteenth	2 2	2 2

Nitsch uses 5 to 1 day cord; 0.1 to 0.15 gm. of cord to 2 c. c. physiological solution. Treatment lasts but ten days, two injections daily. Each patient receives 2.5 gm. of cord during treatment. Very severe cases are treated sixteen days.

Of 1,424 cases treated, 6 died=0.42 per cent. One case stopped treatment before its completion, and 5 died before fifteen days after completion. These results are very favorable.

Nitsch suggests, as has also been suggested by Babes, the concomitant use of serum therapy with the use of cerebral cortex. Rabic cerebral cortex is shown to be fifty times as virulent as rabic cord.

The technic of the Pasteur method will be more fully considered later (p. 65).

The method in use at the Hygienic Laboratory is given on page 68.

(4) *Fixed virus attenuated by heat.*—This method advocated and used by Puscariu and Vesesco at Jassy, and by Babes, seems to be merely a more difficult manner of attaining the same end accomplished by the desiccation and dilution methods. It is said, however, to have been more recently adopted in Japan. Babes defends the method inasmuch as his mortality since employing it has been very low. He combines this method in severe cases with the use of antirabic serum.

It is claimed that by heating emulsions of fixed virus to 80° C. for fifteen minutes the organism of rabies is killed, but that a relatively thermo-stabile substance, capable of provoking immunity, is left unaltered. Paralytic symptoms (see *ill effects from treatment*) are said to be more frequent after this form of treatment.

(5) *Fixed virus modified by partial digestion.*—This so-called Italian method consists in exposing the virus before injection to the action of artificial gastric juice. It is claimed that in this way an actual attenuation is accomplished; not merely a numerical reduction of the exciting organisms, and that virus so treated can not be restored to its original virulence by passage through animals.

(6) *Fixed virus acted upon by bile.*—Has been suggested for treatment following the experiments of Frantzius, Vallée, and Solomon, and Kraus, but so far as known has not been applied to man.

(7) *Fixed virus acted upon by glycerin.*—While glycerin has the power to conserve rabies virus in an active condition for a month or more (Calmette), on prolonged exposure this virulence is suddenly lost (two and one-half months, Loir; five months, Rodet and Galavielle (107)), although the immunizing power is, to some extent at least, retained. Rodet and Galavielle note the immunizing action of rabic nerve matter which has lost its virulence through prolonged conservation in glycerin. Rarely is this immunity sufficiently substantial to withstand subdural-test inoculation with fixed virus,

but it constantly resists subcutaneous inoculation. Galavielle and Martin (38) tested the same immunity with the subdural inoculation of street virus in rabbits and found it to be considerably more marked than when tested in the same way with fixed virus, since out of 13 rabbits subcutaneously immunized only 6 developed rabies, and 2 of these only after prolonged incubations. They suggest the possibility of glycerinized virus treatment for persons exposed to rabies.

(8) *Fixed virus acted upon by antirabic serum.*—Marie (67) claims that by treating, at least for the preliminary injections, with fixed virus partially neutralized with antirabic sheep serum *in vitro*, a rapid immunity is conferred, inasmuch as the lysis ordinarily accomplished in the tissues is partially effected *in vitro* by the antirabic serum. He claims that in this way virus of high immunizing power, but of diminished infectious properties, can be administered. He states that since 1904 nearly three hundred patients have been treated in this manner with excellent results.

The method is described as follows:

One gm. of the bulb of a passage rabbit (f. v.) is rubbed up with 9 c. c. of weak broth or physiological salt solution and the emulsion strained through cloth. To 2 c. c. of this decimal emulsion is added 4 c. c. of antirabic sheep serum, previously heated for thirty minutes at 56° C. The 6 c. c. mixture, containing an excess of virus (i. e., unneutralized), is injected into two places under the skin of the abdomen. The same injections are repeated on the three following days, after which the patient receives inoculations of dried cord, beginning with that of the sixth day. The serum used was of insufficient strength to neutralize the virus in the proportions given.

This treatment he considers especially applicable to severe bites and to belated cases. The mortality in treated persons is said to have been lowered since the introduction of this treatment.

(9) *Fixed virus acted upon by carbolic acid.*—Fermi (31) has had better success by this method, he claims, than is attained by the Pasteur method. He claims that the Pasteur method does not invariably protect the Muridæ against one and two day cords and claims that a virulent strain of fixed virus might cause rabies in man, and that his 5 per cent emulsion of fixed virus acted upon by 1 per cent carbolic acid is free from these objections.

(10) *Fixed virus acted upon by mechanical disintegration.*—The experiments of Barratt and of Heller have shown that by prolonged rubbing in the frozen condition the infectiousness of fixed virus can be destroyed, and, further, that this destruction is not due to the low temperature but to an actual breaking up of the micro-organisms, which is readily effected in the congealed state. While this comminuted material is not infectious and can not therefore reproduce the disease, it is toxic and capable of inducing a prolonged

cachexia, marasmus, and sometimes paralysis, which can only be attributed to a poison contained within or elaborated by the microorganism. Heller has also shown that animals may be immunized to this substance by the administration of progressively increasing doses, and that this immunity is active also against rabies infection. Of 10 rabbits so treated, only 1 developed rabies after subcutaneous inoculation with passage virus, while all of the control animals died of rabies. This method has not thus far been applied to the treatment of man.

Serum therapy.—Great hopes that a cure for developed rabies was at hand were entertained when it was learned that the blood of immunized persons and animals possessed the power to destroy the infectious principle of rabies virus *in vitro*. It was hoped to establish an immediate passive immunity, comparable to that caused by antidiphtheric serum, as distinguished from the delayed active immunity conferred by the older methods and comparable to that induced by vaccination against smallpox. Although encouraging results have been obtained in the lower animals with respect to their immunization with antirabic serum, with regard to its curative effects serum therapy has been very disappointing. Marie advises the dried and pulverized serum as a local application to wounds caused by animals suspected of rabies, and employs the serum in the fresh state to partially neutralize the fixed virus used in the first injections of the course of antirabic treatment as above given in detail. The properties of antirabic serum are discussed under the subject of Immunity.

Who shall receive the Pasteur treatment?—Persons who have been bitten by rabid animals or who have had open wounds or scratches contaminated with the saliva of such animals should receive the treatment. At times the question has been raised whether persons who have drunk of the milk of rabid cows should be subjected to the preventive inoculations. While the possibility of infection by this means is extremely remote, there is some evidence that the virus can sometimes be present in the milk. On the other hand, infection through the intact mucosa of the alimentary tract is highly improbable, since the action of the gastric juice is destructive to the virus.

Persons bitten by animals presenting symptoms strongly suggestive of rabies should receive the antirabic treatment whether or not the suspicion is confirmed by histological examination and pending the result of the inoculation test, if such is made. The necessity for treatment arises from the fact that rabies is really much more prevalent among animals than is generally suspected, and too much valuable time will be lost by delaying treatment for the result of animal inoculation.

Persons bitten by animals not showing any of the symptoms of rabies are not exempt from the necessity for treatment until the biting animal, which should be closely confined and carefully observed for certainly not less than two weeks, shall be shown to be free from the disease, since the saliva is known to be virulent sometimes several days before the onset of the symptoms; four to five days according to Roux and Nocard (110); Zagarro (120), twelve days.

In this connection it is deemed pertinent to repeat that the histological examination is conclusive only when positive; that Negri bodies (p. 33) when demonstrable in the central nervous system are positive indication, according to present knowledge, that the animal was rabid; but the failure to find them does not exclude rabies, since they may not have developed to a demonstrable condition at the time the animal was killed, or they may have escaped detection through technical imperfections, or, finally, they may not be present even in well-developed rabies in the portions examined.

The results of the Pasteur treatment and its modifications.—In order to determine the value of the method it is necessary to compare the mortalities of treated and untreated persons. We believe that no exact comparison can be made, but that there is ample evidence to show that an enormous saving of life has resulted from the timely administration of the treatment.

The inaccuracy of this comparison arises from the difficulty of determining its latter factor, namely, the mortality of untreated persons. It is hardly to be supposed, even in countries where official supervision is the most intimate, that every dog bite or even every case of human rabies has come to official notice, or that the existence of rabies in the biting animal can have been accurately determined in every case. The severity and site of the injury must also be considered as a variable and in many cases unrecorded quantity. Sources of error are found also in the determination of the mortality of treated persons, but to a much less extent. Patients can, for the most part, be accounted for during the year following the treatment; and, thanks to the present state of knowledge, the diagnosis of rabies in the biting animal can be accurately established in many cases. Then, too, the arrangement of the records of patients into categories (see p. 70) according to the basis of diagnosis lends additional accuracy to treatment statistics.

Estimations of mortality in untreated persons.—In Prussia statistics are collected with as much care, probably, as in any other country. The recent statistics of Doebert (1909), based on official records, show a mortality of 14.8 per cent in 122 untreated persons who were significantly bitten by rabid animals within the period from 1902 to 1907. Cases of insignificant bruises, etc., and of bites by animals not proved to have been rabid were excluded in the computing of these statistics. He quotes the estimates of other observers, as follows: Horsley, 16 per cent; Reifer, 15 per cent; Dujardin-Beaumetz, 14 per cent; Högyes, 15 to 16 per cent.

Schüder (112) collected statistics of 14,959 persons bitten by rabid or suspected animals and untreated, and found that the mortality varied greatly in different countries, but averaged 8.9 per cent. We should of course expect Schüder's figures to be lower than those of Doebert, since the character of the injury and the proof of rabies in the biting animal were not considered in the former's estimate.

Marx (1904) thinks that the older figures were, as a rule, too high, and that a mortality of 6 to 10 per cent of untreated bitten persons would approximate the truth.

In the United States there are no available statistics of a general scope upon which a mortality estimate of untreated persons can be based. Among the isolated attempts to form such an estimate is that of Brawner (15), who followed in the newspapers such cases of bites and deaths as were reported to have occurred within the Southern States. Of 80 persons untreated, except by the "madstone," there were 16 deaths reported=20 per cent, while of 170 treated at the Pasteur Institute only 1 died.

Mortality in treated persons.—Doebert reports a mortality of 1.3 per cent among 1,741 persons having received *significant bites* from *rabid* animals and taken the Pasteur treatment during the period of 1902–1907. Only 0.85 per cent, however, died more than fifteen days after the end of the treatment, and hence are regarded as flat failures of the treatment, for which no claim is made that immunity can be invariably established by it before that time.

In general, it may be stated that the total mortality of bitten persons having received the antirabic inoculations is pretty constantly about 1 per cent, of which one-half could not, from the nature of the treatment, have been expected to live, on account of the short time permitted for the establishment of immunity. In other words, 0.5 per cent die of rabies within fifteen days after the completion of the treatment that can not be charged to its failure, but to a want of time to establish immunity.

Remlinger (97) gives the mortality of treated persons in twenty institutes, which shows an average mortality of 0.5895 per cent occurring within fifteen days of the end of the treatment. These figures cover various periods of time, the latest year reported being 1903.

Results are always poorest where the bites are the most severe, for example, where wolves abound, and where from the lack of transportation facilities a long time has elapsed before the patient is received for treatment. Results are better as the older formulæ of treatment are replaced by those in which more virulent material is administered.

This is illustrated by the experience at Cracow (Nitsch (80)) where, with the use of a mild form of treatment lasting but ten days, the mortality was 1.05 per cent; but since the adoption of a more intensive treatment it has been reduced to 0.42 per cent; or, eliminating the deaths occurring within fifteen days after treatment, nil. There were 1,424 cases treated.

These two facts taken together, namely, the increased mortality with severe bites and the decreased mortality with greater intensity of treatment, furnish a powerful argument for the reality of rabies and for the innocuousness and the value of preventive inoculations.

THE TECHNIC OF CERTAIN OPERATIONS CONCERNED IN THE MANAGEMENT OF THE ANTIRABIC LABORATORY.

(1) *Examination of suspected material for evidence of rabies.*—This material consists ordinarily of the head of some animal, oftenest the dog. It should be shipped packed in ice if practicable; if not, it may be wrapped in cloths soaked in bichloride of mercury or other germicidal solution. For microscopical examination, material may be sent already fixed and placed in weak alcohol. For inoculation tests, the fresh material or the medulla placed in glycerine is suitable.

Many methods have been employed for the demonstration of the Negri bodies. The individual examiner must determine the method which gives uniform results in his hands.

In our experience, the rapid methods, while often giving brilliant results, and undoubtedly time saving when positive, have been less reliable in the end than the sections. It is our practice to make smears from Ammon's horn and examine them immediately. Failing in the search for the bodies, other portions of the brain are examined: the cerebral cortex, cerebellum, and medulla. If the search is still unsuccessful, sections are made in which the cells are found in their normal relations and the search is facilitated by their proximity.

Both histological examination and inoculation tests should be made, at least until one's technic is assured, and even then the inoculation tests should always be applied when the histological examination is negative or doubtful. For this purpose the medulla should be preserved in glycerine pending the result of the histological examination.

Removal of the brain of animals for examination.—The head is held by an assistant by means of a pair of lion-jawed forceps attached to the snout and held in one hand, while the other hand, protected with several folds of a towel wet with antiseptic solution, is employed to steady the head at appropriate points. The skin is divided down the median line and the muscles dissected away from the bone. The outer tables of the skull are then sawed through in a line running from the foramen magnum to the frontal sinus on each side. Division of the bone is completed with the chisel and mallet, and the calvaria torn off. The membranes are divided and the brain removed by shelling it out whole with closed curved scissors, cutting off the cranial nerves as they appear. Where the tentorium is osseous, it may be divided with bone shears if it does not pull out readily. It is advisable to remove the brain intact if possible in order that the different portions, especially the hippocampus major, may be recognized and specimens taken from them.

Locating the hippocampus or cornu ammonis.—The brain is placed base upward and the temporal lobe lifted outward away from the median line until the cornu comes into view as a long cylindrical whitish body tapering at its uppermost end. It is further identified by sectioning it transversely when the characteristic concentric light and dark zones will be seen. This peculiar structure also serves to distinguish the cornu if a horizontal section is made with a sharp knife through the hemisphere. The cornu can be recognized even in softened brains if the parts be not too much disturbed by handling.

Locating the plexiform ganglion.—This may be accomplished by following up the course of the pnumogastric nerve, which will be found in relation to the carotid artery, to a point one-half inch or less from the base of the brain where the enlargement will be encountered and may be removed for sectioning and examination to demonstrate the changes described by Van Gehuchten and Nelis.

Staining Negri bodies.—We have found the Mallory eosinmethylene-blue method, early recommended by Williams and Lowden, to be the most dependable one. It may be shortened considerably without serious detriment.

Smears are made on slides or cover glasses by crushing a small section of the brain matter between two of them and drawing them out under gentle pressure to produce a fairly thin film.

(*a*) Fixation in Zenker's solution for fifteen minutes.

(*b*) Wash in tap water.

(*c*) Ninety-five per cent alcohol tinted with iodine.

(*d*) Absolute alcohol five minutes.

(*e*) Five to 10 per cent watery solution of eosin (Grubler, W. g.) five minutes.

(*f*) Stain in Unna's polychrome methylene-blue two to three minutes.

(*g*) Wash in water.

(*h*) Differentiate in 95 per cent alcohol.

(*i*) Blot off, dry, and examine with oil immersion lens.

Sections.—Fix thin pieces in Zenker's solution for two and one-half to three hours. Wash well. Ninety-five per cent alcohol, two changes, one hour each. Absolute alcohol, two changes, one hour each. In cedar oil until cleared. Cedar oil and paraffine equal parts, one hour. Paraffine, two changes, one hour each. Embed, section and stain as above, but increase time somewhat, especially in iodine-alcohol where the sections should remain until the mercury granules disappear.

Negri bodies stained in this way take a magenta color, easily distinguished from the bright eosin tint of the red-blood corpuscles, and dark-bluish granules are distinguishable in their interior. The nerve cells and the nuclei of the glia and the endotheliad cells are blue (*see* Pl. I).

This method of staining is less successful following more rapid methods of fixation, but fairly good pictures are obtained with sections fixed in acetone.

The material is placed in acetone for forty-five minutes, then in several changes of paraffine; embedded and sectioned. The iodine-alcohol step is omitted.

Williams secures beautiful specimens by a modification of Mann's method, which has the advantage of being rapid and easy. Our results with this modification, while sometimes very satisfactory, have been irregular and have necessitated in some instances a resort to the longer methods. The method is as follows:

For smears: Make smears as usual. Fix for ten seconds in neutralized methyl alcohol (500 c. c. alcohol+0.25 gm. Na_2CO_3), to which 0.1 per cent of picric acid has been added. Stain over the flame until steamed in methylene blue (sat. alc. sol.), 30 c. c.; fuchsin (sat. alc. sol.), 5 c. c.; distilled water, 300 c. c. Wash, blot, and examine.

Frothingham's method of making touch preparations is designed to preserve the natural relations of the cells, and in this way facilitate the search for the Negri bodies.

Other methods are those of Harris (42), which is very good; Giemsa, as employed by Negri (74); Neri (75), a modified Gram's stain; Hematoxylin and fuchsin; Lentz (58), eosin and Lœffler's alkaline methylene blue; Borel's eosin-methylene blue-silver oxide, given in detail in Laveran and Mesnil's "Trypanosomes et trypanosomiasis," Paris, 1904, pages 10 and 11; and numerous others devised and employed by various observers. Bohne (13) gives a number in full.

Heyman (41) reports no results of practical value with the use of the so-called rapid methods, stained or unstained smears, Negri, Luzzani, or Van Gieson's method, dark field illumination and frozen sections. He uses paraffine sections and Lentz's modification of Mann's method almost exclusively.

The search for the rabic tubercles of Babes and the ganglion changes of Van Gehuchten and Nelis involve only the ordinary histological procedures which require no special descriptions.

(2) *Animal inoculations.—Animals used:* For this purpose rabbits have the advantage of exhibiting the symptoms in a more characteristic manner than the smaller animals, and the disadvantage of having a longer incubation period, which is rarely less than fourteen days, and frequently much longer.

Guinea pigs are much cheaper and exhibit symptoms several days earlier than do rabbits, but frequently the disease is of such short duration that little opportunity is afforded for observation.

Rats have been used to some extent, but they require a special holder and are difficult to handle. They are suitable for the intramuscular injection only.

In default of rabbits and guinea pigs, in emergency almost any small mammal may be utilized for subdural inoculation.

Material.—Portions of the medulla, or failing in this the cerebral cortex, are the most suitable for inoculation. In the case of man where autopsy is denied, the saliva or aspirated cerebro-spinal fluid may be used; but the results have significance only when positive, since these secretions are frequently noninfectious in positive cases.

Fresh material is of course preferable, but old and putrefied material may still be employed if emulsified in glycerine and permitted to stand twenty-four to forty-eight hours before its injection. Some of the emulsion should be kept for further inoculation in case acute death occurs in the first inoculated animals. Or the material may be rubbed up with sufficient quantity of 1 per cent solution of carbolic acid and injected.

Method of inoculation.—With fresh material the subdural inoculation is to be preferred, but with putrid material deep injection into the muscles of the neck will result in nearly as large a percentage of "takes," with fewer deaths from intoxication or sepsis. The incubation period, however, is likely to be prolonged.

The emulsion is made by rubbing up a small piece of brain tissue (size of a pea) in a sterile mortar, and then adding a few cubic centimeters of salt solution and reducing it to a uniform consistency.

Subdural inoculation.—This method of inoculation is performed most satisfactorily by etherizing the animal. Make a one-fourth inch incision through the skin just back of the eyes and to one side of the median line, draw the skin back over the parietal bone, and with the aid of a stylet or a small trephine or jeweler's drill effect an opening through the skull large enough to readily admit the needle, which, attached to a small syringe, is now passed through the opening and 0.2 c. c. of the emulsion injected. The needle should be bent forward one-fourth inch from its tip so that the fluid may get well under the dura. The skin is then allowed to slip forward and the opening is closed with collodion and a little cotton. The operation requires but a few seconds, and as soon as the animal recovers from the anesthetic it resumes feeding with no apparent inconvenience.

The old method of removing a button is unnecessarily laborious. Sutures are not required, since only a small incision is made, and moreover are sometimes followed by stitch abscesses.

The illustration (Pl. II) shows a convenient form of head holder for rabbits, which is easily constructed.

This method is applicable for routine inoculation of passage animals with fixed virus in the production of antirabic vaccine.

(3) *Removal of the cord.*—The method of Oshida (84) has many advantages over the older method of laminectomy, which it has wholly superseded at the Hygienic Laboratory. As improved by us, it is carried out in the following manner:

The rabbit, when completely paralyzed, is killed with chloroform and nailed to a board, back uppermost, and thoroughly wetted down with an antiseptic solution (1 per cent tricresol). An incision is made through the skin from the forehead nearly to the tail and the skin laid back on each side, the ears being cut close to the head. An area 1 inch wide is seared with a hot iron around the occiput and nuchal region and ear openings, and another across the spine just above the sacrum. The spine is then divided transversely in the center of the seared areas by means of bone-cutting forceps. The neck is dissected loose from the skin, etc., and a large square of sterile gauze is inserted beneath it. The lumbar region is dissected up for a few inches and a similar piece of gauze placed beneath it. Then a piece of telegraph wire about 14 inches long, bent into a handle at one end and having a small wisp of cotton twisted about the other end, is used to push the cord out of its canal. The spine is steadied by a pair of lion jawed forceps (Pl. III).

An assistant catches the cord with forceps as it emerges from the cervical opening and lifts it out. The spinal nerves are torn off during this procedure and the membranes stripped off, leaving a clean sterile cord. A silk ligature, with one long end, is placed around the upper end and another just below the middle of the cord, which is then cut in two just above the lower ligature. A small piece is cut off the lower end of the upper portion and placed in a tube of bouillon, which is incubated as a test of sterility. The cords are then hung in a drying bottle (see illustration). All manipulations are under aseptic conditions.

Drying the cord.—The cords are hung in the drying bottles (Pl. IV) by their attached ligatures, which are retained by the cotton plug. Sticks of caustic potash are previously placed in the bottom of the bottle, as shown. The bottles are properly labeled with the number of the rabbit and the date, and placed in the incubator having a constant temperature of 22° to 23° C. This may consist of the ordinary gelatine incubator maintained in a cold room by gas, or one of the more elaborate cabinets designed for this purpose.

Use of the cord.—After one day drying the cord is known as "1-day cord," after two days as "2-day cord," etc. Pieces may be cut off each day and used immediately, in which case rabbits will have to be killed daily in order to have cords of all degrees of virulence always at hand; or, as is the practice at the Hygienic Laboratory, 1-centimeter pieces are cut off daily up to the eighth day and placed immediately in glycerine. Cords in glycerine and kept in a cold place will retain their potency at the point where it was when they were cut, little if any diminished, for several weeks and a constant supply may thus be kept on hand without the daily killing of rabbits. The material conserved in this way is available for shipment and use at a distance from the laboratory. It is first cut into one-half centimeter pieces, each of which serves, when emulsified with 2½ c. c. of salt solution, for one injection.

Titration of the serum of persons or animals for the determination of antirabic properties in vitro.—The conditions of experiments of this kind should always be carefully recorded. The method employed at the Hygienic Laboratory is similar to that used by Marie, and is carried out as follows: Virus is taken immediately after death from the medulla of a series rabbit which has been inoculated subdurally with fixed virus, has shown definite symptoms on the seventh day thereafter, has become completely paralyzed, and has been killed on the eighth day by chloroform. A weighed amount of this virus is rubbed up by hand very finely in a porcelain mortar, and enough 0.8 per cent sodium chloride solution added gradually without interrupting the trituration to make 100 parts. To 1 gram of bulb, 99 c. c. of salt solution would be added. When thoroughly emulsified the fluid is filtered through a paper filter (Schleicher and Schüll No. 588 Faltenfilter). Equal amounts (1 c. c.) are now added to each of a series of small conical test glasses. The serum to be tested is now added to all but one of these glasses in different amounts (e. g., 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0 c. c.). The mixture is stirred up with a sterile platinum needle, and placed in the cold room or ice box for twenty-four hours. During this time they should be stirred up once or twice. Now sufficient salt solution is added to each glass to bring the total quantity contained by each up to the maximum contained by any, including, of course, the control to which no serum has been added. Equal amounts (0.2 c. c.) are now injected into a series of test animals, rabbits or guinea pigs, corresponding to the series of dilutions. Accuracy of dosage is secured by making the injections intracerebrally rather than subdurally. The antirabic property of the serum is indicated by the period of incubation and of death of the animals.

TREATMENT OF PATIENTS BY THE HYGIENIC LABORATORY.

Patients coming to the Hygienic Laboratory for the preventive inoculations are treated according to the following formula, which corresponds closely to that in use at Berlin. All formulæ are more or less arbitrary, and this one is no exception. It was adopted for the following reasons:

(1) That cords dried for a longer period than eight days are devoid of infectious properties and presumably are also lacking in immunizing properties. Their use may therefore be regarded as a waste of valuable time, since the average incubation is all too short to consume any part of it with superfluous procedure.

(2) On the other hand, the use of cords of greater virulence than eight-day cord to begin with was set aside as having a less widely demonstrated innocuousness and less authoritive support, although we believe that it can be done without danger. So far as our experience has gone, no advantage would have accrued, since up to the present time (January, 1910) no deaths have occurred after treatment.

(3) Following the custom established by Pasteur, the injections of more virulent material are spaced through the treatment and increased in number near its termination.

Scheme for mild treatment.

Day.	Cord.	Amount injected.			Day.	Cord.	Amount injected.		
		Adult.	Five to ten years.	One to five years.			Adult.	Five to ten years.	One to five years.
	Injections.	*c. c.*	*c. c.*	*c. c.*		*Injections.*	*c. c.*	*c. c.*	*c. c.*
1	8-7-6=3	2.5	2.5	2.0	12	4=1	2.5	2.5	2.5
2	5-4=2	2.5	2.5	1.5	13	4=1	2.5	2.5	2.5
3	4-3=2	2.5	2.5	2.0	14	3=1	2.5	2.5	2.0
4	5=1	2.5	2.5	2.5	15	3=1	2.5	2.5	2.0
5	4=1	2.5	2.5	2.5	16	2=1	2.5	2.0	1.5
6	3=1	2.5	2.5	2.0	17	2=1	2.5	2.0	1.5
7	3=1	2.5	2.5	2.0	18	4=1	2.5	2.5	2.5
8	2=1	2.5	1.5	1.0	19	3=1	2.5	2.5	2.5
9	2=1	2.5	2.0	1.5	20	2=1	2.5	2.5	2.0
10	5=1	2.5	2.5	2.5	21	2=1	2.5	2.5	2.0
11	5=1	2.5	2.5	2.5					

Scheme for intensive treatment.

Day.	Cord.	Amount injected.			Day.	Cord.	Amount injected.		
		Adult.	Five to ten years.	One to five years.			Adult.	Five to ten years.	One to five years.
	Injections.	*c. c.*	*c. c.*	*c. c.*		*Injections.*	*c. c.*	*c. c.*	*c. c.*
1	8−7−6=3	2.5	2.5	2.5	12	3=1	2.5	2.5	2.0
2	4−3=2	2.5	2.5	2.0	13	3=1	2.5	2.5	2.0
3	5−4=2	2.5	2.5	2.5	14	2=1	2.5	2.5	2.0
4	3=1	2.5	2.5	2.0	15	2=1	2.5	2.5	2.0
5	3=1	2.5	2.5	2.0	16	4=1	2.5	2.5	2.5
6	2=1	2.5	2.0	1.5	17	3=1	2.5	2.5	2.5
7	2=1	2.5	2.5	2.0	18	2=1	2.5	2.5	2.0
8	1=1	2.5	1.5	1.0	19	3=1	2.5	2.5	2.0
9	5=1	2.5	2.5	2.5	20	2=1	2.5	2.5	2.5
10	4=1	2.5	2.5	2.5	21	1=1	2.5	2.5	2.0
11	4=1	2.5	2.5	2.5					

Treatment at a distance from the laboratory.—This has been rendered possible in recent years through the use of glycerinated virus. Glycerine may be added to the emulsion, and this shipped in a special container; or the rabbit cord itself, cut into pieces representing single doses, may be sent in bottles containing glycerine. These pieces of cord must be emulsified by the physician using them. This latter method has been used in shipping virus from the Hygienic Laboratory to state health officials, upon their requests to the Surgeon-General, United States Public Health and Marine-Hospital Service, Washington, D. C. It necessitates some technical skill in its preparation and use, and for this reason it is stipulated that the virus shall be administered under the immediate supervision of the state health authorities.

The bottles containing the virus are accompanied by the following directions and copies of the schemata of injections:

Directions for the use of rabies virus shipped from the Hygienic Laboratory, U. S. P. H. & M. H. S., Washington, D. C.

The virus should be kept, until used up, in an ice box or other cold place. This material is perishable, and must not be kept on hand for future use. Each bottle contains the number of doses required, until further shipments are made, of cord dried for the number of days indicated on the label. Further shipments of cord to complete treatment already begun are made without further request.

Dose.—Each small section of cord (about one-half cm.) constitutes one dose.

The following equipment is necessary for making and using the emulsion:

Physiological salt solution.
Alcohol.
Absorbent cotton or gauze.
Glass or porcelain mortar and pestle (capacity, 10 to 20 c. c.).
Thumb forceps.
Hypodermatic syringe (at least 3 c. c. capacity) with large needle.
Glass pipette, 5 c. c., graduated at least to 0.5 c. c.
Small conical test glass or beaker or other small container.

These must be sterilized, and then the instruments, etc., rinsed in sterile salt solution. Aseptic technique throughout.

To make the emulsion.—Remove one section of cord from the bottle with the thumb forceps, and rinse it free of glycerin with sterile salt solution in the small glass container, place it in the empty mortar, and, without the addition of any fluid, rub up as finely as possible. Then 2½ c. c. of the salt solution are gradually added by means of the pipette, taking care between the additions of salt solution to rub to a uniform consistency. Draw the full amount of the emulsion into the syringe.

To use.—Scrub the skin at site of inoculation with alcohol and inject the emulsion into the subcutaneous tissue, being careful not to injure muscular layers or visible veins. Alternate successive injections on the two sides of the anterior abdominal wall.

For the schemes of injections see accompanying sheets.

A record should be kept of each case, giving essential particulars concerning the patient, his injury, treatment, and subsequent history, with dates; and also concerning the biting animal and the basis upon which the diagnosis of rabies was made in it.

For the purpose of accurately determining the value of the Pasteur or other preventive treatment of rabies, the plan adopted at the Pasteur Institute at Paris should be followed.

Patients receiving the treatment are classified according to the evidence of rabies in the biting animal as follows:

Group A. Rabies demonstrated by animal inoculation.

Group B. Rabies diagnosed by a competent veterinarian.

Group C. Cases in which, from the evidence at hand, it is supposed that the biting animal was rabid.

To this may now be added another group in which the diagnosis is based upon the demonstration of Negri bodies.

Contraindications for treatment.—No specific contraindications exist. It is true, in a small percentage of syphilitics, alcoholics, and neurasthenics, and probably in some apparently healthy persons, a transient paralysis may occur or even death from ascending paralysis. These cases, however, are not definitely proven to be due to the treatment, and in any event they are outweighed by the total saving of life. Pregnant women have taken the treatment without disturbance. A boy severely affected with chorea seemed improved rather than otherwise by the treatment.

Ill effects from the treatment.—The statement is frequently seen that the Pasteur treatment is without danger *per se*. This statement is not strictly true. There are on record a small number of fatal cases in which the treatment was apparently responsible, and a larger, but still small, number in which a serious but temporary paralysis occurred during or immediately after the treatment, and could reasonably be attributed to it.

These cases can not be considered as seriously impairing the value of the treatment any more than deaths from the administration of anesthetics, fat embolism in the reduction of fractures, or from anaphylaxis in the administration of diphtheria antitoxin, respectively, occurring in a very small percentage of cases, can be considered to favor the general abandonment of these procedures. The rarity of this accident will be appreciated when we consider that Remlinger (98), upon searching the records of 107,712 persons that had received the treatment, found but 40 cases of treatment paralysis, 2 of which resulted fatally. Subsequent reports have increased the total number of reported cases to about 76 with 2 additional fatal ones (Müller, Pampoukis, Fabricius, Jones).

The principal explanations that have been advanced for the phenomenon of treatment paralysis are as follows:

(1) That the symptoms are due to the street virus received from the biting animal which becomes so modified by treatment that the disease runs an atypical and usually a mild course with recovery as a common result. This view was supported by Roux, Laveran, and a

majority of the early observers of the condition. Against this view are the facts that the accident occurs as frequently, if not more so, after slight injuries as after severe bites; that several cases have occurred in which the biting animal was not rabid (Nedrigailoff and Ostrjanin (73), Fabricius (30), Torini, see Müller, p. 269); that pain at the site of injury is rarely experienced; that the period of incubation is too short for infection with street virus; and finally that paralytic rabies is usually considered an intense rather than an attenuated form of rabic infection. Babes and Marinesco (6) describe the lesions, which are not those of rabies, in a fatal case, and state that the animals inoculated with the brain matter failed to take rabies. Josef Koch (52) expresses himself in favor of the view that these cases are abortive street virus infections.

(2) That the condition is due to infection introduced with the virus, in the form of contaminating microorganisms, during the treatment. Several observers have claimed to have isolated such organisms, but they were of different kinds in each case, while the symptoms were the same. Again, myelitis of bacterial origin is a very fatal disease, while these cases of treatment paralysis having symptoms of myelitis, or at least congestion of the cord or bulb, recover spontaneously in the great majority of instances.

(3) That treatment paralyses are instances of fixed-virus infection. The early appearance of the symptoms in many cases is against this view, since virulent emulsion is not commonly injected during the first days of treatment, and the paralytic phenomena have been known to appear from the first to the seventh day after the administration of the first virulent injection.

(4) That the rabies "toxin" is the cause of the paralysis. While some authors (Babes, Remlinger, Marie, etc.) seem confident of the existence of such a substance *in active form*, discussion elsewhere given (p. 39) appears to leave room for reasonable doubt. However, the transient nature of the disorder is adduced as an argument in favor of an intoxication.

(5) That hysteria is competent to explain the phenomena. Evidently this is not so, since the stigmata of hysteria are not commonly found and the sphincteric involvement speaks against it.

(6) That the phenomenon is one of anaphylaxis, due to the repeated injection of a foreign proteid represented by the rabbit cord employed for the preventive inoculations. This idea, advanced by Harvey and McKendrick and entertained by Müller, seems to have much to recommend it. It is stated that these phenomena have never followed treatment with diluted fixed virus as used by Högyes, and it is in this form of treatment that the proportion of nerve substance to active virus is at a minimum.

Marinesco adverts to the cytotoxic action of foreign nerve substance as a possible explanation of the phenomena.

Whichever, if any, of these explanations may prove to be the true one, it is evident that a special individual susceptibility must exist, otherwise the paralysis would occur much more frequently among those who have received the preventive inoculations.

Certain authors have claimed that in the majority of their cases of treatment paralysis, a neurotic personal or family history could be traced, which might argue a peculiar susceptibility of the nervous system. Alcoholism and sudden chilling of the body are likewise supposed, and apparently with good reason, to render the nervous system hypersensitive to the exciting cause, whatever that may be. The phenomenon is said not to have occurred after treatment by Högyes's method, and to have been most common after treatment according to the Roumanian practice.

The symptoms usually appear from a week after the commencement of the treatment to shortly after its termination. There is usually insomnia, headache, and a feeling of numbness in one or more extremities, oftener in the lower. Sometimes pain or itching at the site of the injection is complained of. The affected member becomes weak and finally paralyzed and the paralysis extends in a day or two to other portions of the body. The patient becomes helpless. The rectum and bladder are frequently more or less paralyzed. Bulbar symptoms are not rare; there may be difficulty in swallowing, but there is no hydrophobia. The special senses are frequently affected, especially vision. The paralysis is flaccid, the reflexes being diminished or lost as a rule. The Babinski phenomenon is sometimes observed. The condition of the patient may become most alarming, but usually a rapid improvement takes place and health is regained in a few weeks, although months are sometimes required for complete restoration. In some instances the condition is apparently a myelitis; in others the peripheral nerves appear to be involved. Pfeilschmidt records a case in which the paralysis involved chiefly the facial muscles.

No special treatment is indicated. The preventive inoculations should be discontinued, although in several reported cases they have been persisted in without apparent detriment.

Some benefit has been claimed by Remlinger from the use of antirabic serum. Cystitis, bedsores, and inhalation pneumonia are to be guarded against.

There appears to be no criterion by which this condition can be differentiated from paralytic rabies at its inception, but a few observations may be of value in this connection: The initial fever is usually slight, that in rabies is higher; the pains are not referred to the bitten parts, but rather, if at all, to the site of the injection;

the period of incubation is usually too short for that of true rabies; the mental symptoms of rabies are usually absent. The termination is by recovery as a rule, frequently at a surprisingly rapid rate. sometimes much longer deferred.

Among 180 persons treated at the Hygienic Laboratory there occurred one case of treatment paralysis in a man who had had meningitis in childhood. The paralysis was rapid in its progress, and the patient's condition became rather alarming. but he made a good although somewhat tedious recovery. (For references to treatment paralysis see Bibliography Nos. 4, 6, 16, 26, 30, 37, 41, 43, 44, 48, 50, 52, 57, 72, 73, 87, 90, 93, 98, 106, 109.)

Other ill effects from the treatment are, as a rule, of minor importance. The patient usually complains of some discomfort at the site of inoculation, and may be nervous or sleepless, or, on the other hand, drowsy for the first few days. Except the local discomfort, there is no reason to think that these symptoms are specific manifestations of any action of the virus. They are probably psychical manifestations induced by the novelty of the situation. A local reaction of the tissues to the virus is commonly observed in our experience during the second week. Rarely it is observed during the entire treatment. It seems to have no relation to the potency of the virus used. It has seemed to us that children and thin people exhibited it less markedly than others. So far as we know no relation between the local reaction and the establishment of immunity has been worked out. We regard the phenomenon as a manifestation of hypersusceptibility to foreign nerve tissue. Patients have been observed to develop the reaction simultaneously in several inoculation sites after about a week's treatment, during which none had appeared. Marie (66) mentions indurations following the second of two injections, seventeen days apart, and due to the wide separation of the species (dog and rabbit). The reaction consists of a slightly raised and edematous area from 1 to 3 inches in diameter with an erythematous blush occurring around the needle puncture and appearing several hours after injection. There is usually considerable itching and some pain and tenderness. The appearance is shown in Plate V. This subsides in the course of a day, leaving a palpable " knot " under the skin, which is slowly absorbed. The lymph nodes associated with the region are commonly a little enlarged and tender.

Abscesses have not been observed at the Hygienic Laboratory, although they were reported some time after treatment in some marines who came from Panama for treatment. A considerable number of these men also developed malarial attacks. It has been observed that dormant malaria appears to be awakened to activity by the treatment. However, this condition is so frequently observed in persons recently removed from the Tropics that a recrudescence of malaria can hardly be definitely attributed to the treatment.

Various skin rashes are sometimes observed. These have in our experience taken the form of mild urticaria, which disappeared in a few days.

SUPPRESSIVE MEASURES AGAINST RABIES.[a]

The suppression of rabies wherever it exists is worth undertaking seriously from the view point of the sanitarian, the dog owner, and those who own valuable stock. The annual toll of human death is not very great, but a single death from a terrible disease which is preventable is a reproach to the sanitary administration. The loss of time and money to those exposed persons who are obliged to take the Pasteur treatment, frequently through no fault or neglect of their own, is very considerable. Their mental suffering can not be expressed in dollars and cents. During 1908 at least two large hunt clubs were obliged to destroy their entire kennels because of infection with rabies, and many hundreds of valuable dogs were sacrificed for the same reason. The loss of stock—cattle, horses, sheep, and swine—from rabies mounts up to thousands of dollars annually in some areas of the United States.

The measures by which rabies can be exterminated, or at least reduced to a minimum, are well known. It is their application which causes the difficulty, and the crux of this difficulty is popular sentiment and popular apathy. The average citizen either does not believe in the existence of rabies, or takes no active interest in the matter until a personal experience affects his pocketbook or his personal comfort, and then the active interest which he suddenly develops extends no further than his personal affairs. Measures to be effective must be state wide in their application, and a uniformity of legislative and executive action in contiguous States is necessary if any lasting benefit is to be secured.

The history of the fight against rabies in certain sections of Europe is especially instructive in this connection. Great Britain, an island, had little difficulty in completely eradicating rabies where the suppressive measures were administered centrally by the board of agriculture and fisheries in 1897, and in preventing its introduction by adequate quarantine. The same result was obtained in the Scandinavian countries, which are peninsular and have little connection with the mainland. Holland, less isolated, has been less successful, and Belgium, with an extensive French boundary line, still less so.

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[a] This section is practically identical with the Report of the Committee on the Prevention and Suppression of Rabies, approved as a basis for the formulation of regulations by the conference of state health officers with the United States Public Health and Marine Hospital Service, April 30, 1910.

In France the popular opposition to measures against the liberty of dogs, and especially muzzling, has prevented any considerable reduction in the prevalence of rabies except in certain parts where these measures have been well carried out. Germany points with justifiable pride to the extermination of the disease in certain parts where it was formerly very prevalent, but it has been powerless to prevent its persistence and even increase in certain border States, notably Silesia, where infection is continually received from Austria, and where the racial and philological differences in the inhabitants tend to impair the effectiveness of measures.

The States of the Union are for the most part not separated by natural barriers competent to prevent the spread of rabies from one to another. Consequently a uniformity of method must be adopted in order to combat the disease successfully. Antirabic regulations must have an areal distribution as wide as that of the disease, and must persist until, as in England, the disease is completely eradicated. Otherwise local and periodic outbreaks are bound to occur and a condition as bad as, or worse than the present one will ensue.

Without indicating an exact form of regulations for the prevention of the spread of rabies, it may be well to discuss the measures which have proven valuable, what their limitations and the special indications for their employment are. In introduction, it may be stated that the domestic dog and his wild congeners are the principal disseminators of rabies, and it is to prevent the possibility of transmission by these animals that measures must be directed. The wild animals—wolf, coyote, and in certain sections the skunk—are now fast being reduced to negligible numbers in the United States, and consequently require only local measures such as are maintained against any noxious wild beast to prevent their being a danger to man. As has been previously pointed out, the idea entertained by Sime and others that an intermediate host, such as the wild rabbit, is required for the reinforcement of rabies virus is probably erroneous and at any rate without epidemiological significance.

(1) *Destruction of ownerless dogs.*—This is perhaps the most effectual single measure against rabies. It should obtain at all times and in all places, irrespective of the prevalence of rabies. It implies the maintenance of an official dog-catching force and a pound equipped for the detention and humane destruction of dogs. The personnel of the dog-catching force should be carefully selected with regard to probity and good judgment, since these qualities are necessary in those who have an unpopular duty to perform, and the lack of them soon leads to antagonism and even abuse on the part of the public. The ownerless dog, for the purposes of sanitary law, should be defined as a dog unprovided with a collar and license tag of the current year. Impounded dogs may be kept for a few days to permit

of their redemption upon the payment of tax and costs. The sale of impounded dogs is to be discountenanced.

(2) *License fee.*—This is also a permanent measure. All owned dogs should be licensed yearly. Payment of fee secures a license for one year and a tag to be worn continuously, attached to the dog's collar. The tag bears the license number corresponding to the entry on the official register, the number of the year during which it is good, and the name of the place in which it is issued. Changing the form of the tag from year to year renders obsolete tags more readily detectable.

A shield on the collar bearing the name and address of the owner, is a convenience but not a necessity, since these data can be obtained from the register by referring to the license number.

It is suggested in towns of such size and concentration of population that in the opinion of the authorities it would be practicable, to have the tag affixed by the licensing officer or his assistants, in order that it may be securely attached, that the identification of the dog may be complete, that the condition of health of the animal may be observed and its sex ascertained.

The license fee operates in the control of rabies by (*a*) rendering ownerless dogs recognizable by the absence of the tag of the current year, (*b*) reducing the total number of dogs kept, and (*c*) restricting the ownership of dogs to those who have some sense of their value and will, consequently, take better care of them.

The fee should be sufficiently high to accomplish the last two ends. Dog license moneys should be expended in the administration of the dog laws, and not used for unrelated purposes. Unspayed female dogs should be licensed at a higher rate than spayed females or male dogs, with a view to limiting the natural increase of the dog population. Evasion of this provision by deception should be punishable by fine or forfeiture of the dog or the right to keep dogs. In case the licensing officer or his assistant personally affixes the tag, evasion of this fee is precluded.

(3) *Dog owners should be made legally responsible for damage inflicted by their dogs.*—The absurd practice now obtaining in some places of the public assumption of such damages and their defrayal out of the dog license funds by the community should be discontinued. Damage by ownerless dogs may, however, be compensated out of public funds.

(4) *The education of the dog-owning public in matters relating to the care of dogs as affecting the spread of communicable diseases.*—Since public sentiment is such an important factor in the control of rabies, it should be intelligently directed by the authorities charged with this duty through their periodical official publications and leaflets, and cordial cooperation of the officials with the public directly.

(5) *Muzzling.*—This measure should be in constant application wherever rabies exists. It should be abandoned only when the disease has been absent from a region a sufficiently long time to warrant a feeling of security that it has been eradicated and is not merely in abeyance. This time varies much in different regulations now in vogue, but the opinion of many competent observers is that it should not be less than six months. Even this period can not be regarded as absolutely safe, since incubation periods in excess of this time are known to have obtained. Muzzling ordinances for certain months or seasons of the year, when based on a supposed seasonal prevalence, are, in the face of facts, illogical. They should be issued and maintained *pro re nata.* These regulations should involve not only the immediate vicinity concerned, but a sufficient zone about it to insure safety. From the known tendency of mad dogs to wander far, this zone should be commensurately wide.

Muzzling ordinances should prescribe that muzzles be constructed of metal, should prevent biting, should be humane, and permit of the dog's opening its mouth, and should be fitted to the animal, being changed from time to time if necessary, with the animal's growth.

Efficient muzzling stands second only to the destruction of ownerless dogs in efficiency in reducing the prevalence of rabies, and in extensive epizootics takes first place. Unfortunately it is always opposed by a considerable portion of the population, and in many places is not rigidly enforced. Popular enlightenment only can improve this condition. The chief objection against muzzling comes from the dog lover, who claims that it is cruel. There is some justice in this, inasmuch as an ill-constructed or ill-fitting muzzle undoubtedly causes suffering to the animal. A well-made metal muzzle of the basket type, permitting the dog to open its mouth but not to bite, and fitted to its head, is at least as humane as the bit which these critics place in the horse's mouth. Dogs soon become accustomed to wearing them and soon show their appreciation when they are applied, since they know it means an outing for them.

It has been suggested that licensing authorities prescribe the exact type of muzzle to be employed, or even supply the muzzles themselves to secure a suitable article.

Where muzzling is prescribed for the fiscal license year it is recommended that the license tag be firmly affixed to the muzzle itself, so that an unmuzzled dog would be amenable to the rules applying to unlicensed animals.

Muzzling is effective by preventing dogs possibly afflicted with rabies from transmitting the disease by bites, by necessitating the frequent observation of dogs at feeding times, etc., and by indicating an ownerless dog by its absence. When a muzzle is removed from a dog during the period of the ordinance the animal should be restrained by chaining up or by confinement in a suitable inclosure to prevent its running at large.

(6) *Restraint of dogs.*—The compulsory confinement of dogs by their owners on their private premises is a temporary measure employed with success in heavily infected regions. Its duration should not be less than three months and its efficacy increases with a longer period of enforcement. The area of the application should be the same as that in the case of muzzling, upon which measure it may be superimposed if considered necessary. To provide for the exercise necessary to dogs which are thus confined it should be permitted that they be led muzzled in leash at public places. In England special provisions against the night wandering of dogs were found useful.

Special provisions at all times should be made against the running at large of owned female dogs while in heat. Owners of such dogs who allow them at large at such times, even though properly tagged and muzzled, should be made subject to penalty. Female dogs in heat and at large cause the congregation of many dogs together, fights arise among them, and an opportunity for the transmission of rabies occurs if the disease be present in any stage of infectiousness among them. The progeny of such dogs is usually of an undesirable mongrel kind and if not destroyed is soon turned loose to swell the wandering dog population.

(7) *Leading in leash.*—This measure is not advised except the dog at the same time be muzzled. Evasions are so readily and frequently effected that provisions must be made so that if the animal is temporarily released by its owner it will be powerless to inflict bites.

(8) Compulsory notification of the authorities by dog owners and veterinarians of cases of rabies or suspected rabies in dogs or other animals is a useful measure in the early discovery of foci of infection. It should be followed by immediate official veterinary investigation and the destruction of the animal if the diagnosis be confirmed. In case of doubt, especially where a person has been bitten by the animal, effective isolation and observation for at least ten days if necessary should be instituted, to allow time for the development of pronounced symptoms and the confirmation or disproval of the suspicion. The heads of animals so destroyed should be sent for examination to a laboratory maintained by the authorities in charge of antirabic work. Disinfection under official supervision of kennels, stalls, etc., occupied by rabid animals is advisable.

Pending official investigation of suspected cases, the animal should be safely confined, and curative measures should not be attempted on account of the danger of infection.

Bitten animals should also be reported, and in the case of dogs and cats it is wisest that they be destroyed. In the case of valuable stock it should be permitted that they be isolated or quarantined for at least six months from the time of the bite. It should be provided that if dogs, on account of their value, are ever permitted to be quarantined rather than immediately destroyed after being bitten by a rabid animal, this quarantine be carried out by the authorities, or under their supervision, and that the expense be borne by the owner.

The commercial use of any part, including the hide, of an animal killed for rabies must be prohibited unless such parts have previously been rendered innocuous to the satisfaction of the authorities. Proper disposal of carcasses must be enjoined.

Rabies or hydrophobia in man should be made "reportable" throughout the country.

(9) *Quarantine.*—To prevent the introduction of rabies, a quarantine of at least six months should be imposed upon imported dogs. One year would be a safer but scarcely more effectual period, since there are few enthusiasts who would submit to a six months' detention of their dogs, or attempt to import them were this enforced. Quarantine has been effectual in preventing the introduction of rabies in Australia, where the long journey is a strong adjunct, and in England. In the latter country special provisions are made for performing dogs, which permit their introduction into the country under strict police supervision. In Germany dogs may not be moved from one section to another without a certificate from an official veterinarian and other provisions. The expense of quarantine must be borne by the owner.

(10) Immunization of the lower animals has been suggested and even carried out to a slight extent. It is not applicable to dogs, since it is evident that no large proportion of a dog population can be reached by any such method. Valuable dogs should be so cared for that no danger of infection exists. In the event of such a dog being bitten, protective inoculations may be given, if commenced with little delay, to save the life of the animal, but not as a general sanitary measure.

Herds of cattle have been successfully protected by inoculating them; but it is reported that in the method employed—intravenous injection—there was some loss from infection. In our opinion this method of preventing the spread of rabies in the lower animals is not worthy of serious consideration.

CONCLUSIONS.

In conclusion it may be well to summarize briefly what we know about rabies and what we would like most to know.

It is apparent that the disease is quite prevalent in the lower animals and not as rare in man as has been supposed. Exact information as to its prevalence in the United States is not available and its acquisition is necessary. Concerning the species of animals susceptible to experimental infection, we know that all mammals with which man comes in contact can be successfully inoculated, but that those which play a significant part in the perpetuation of the disease are few in number and largely confined to the dog tribe. We now know that outbreaks of rabies in animals are not confined to any particular season of the year.

We have a good deal of data on the pathological findings of the disease, but there are many points concerning its essential pathology that require elucidation. We know that rabies is caused by a living microorganism which invades the nervous system and can be thrown off in the saliva. The exact nature of this organism is still unknown, as is also the nature of its activities and products within the body. The isolation of this parasite, be it animal or vegetable; its cultivation upon artificial media, so that it may be observed under conditions favorable for the determination of its characters, and especially the extraction of its toxic products, if there are such, would seem to offer the greatest opportunity for the discovery of a means of curing the developed disease. Our knowledge of the symptoms of rabies is sufficient to enable us to make a diagnosis in the majority of cases, yet we lack a laboratory test which is applicable during the life of the patient or animal. Inoculation tests enable us to make sure the postmortem diagnosis when suitable material is available, but in practice they require so much time that their value is restricted. The Negri bodies appear to be specific for rabies, but the determination of their exact significance, and their relations to somewhat similar bodies found in other diseases, is still a problem and a most important one.

Until our information concerning the causative organism and the essential pathology of rabies is made definite, our ideas concerning its immunity must necessarily be somewhat vague. We do know, however, that artificial immunity can be conferred during the usual incubation period, and this knowledge has been of great service to us. The possibilities of antirabic serum have not been exhausted by trial. It deserves further investigation, but our present data do not warrant us to expect very much benefit from this source. The treatment of developed rabies by means of drugs, while it has received considerable attention in the past, perhaps because of the discouraging outcome of the earlier attempts at treatment, has received but little attention in recent years. In view of the facts that the balance of evidence leans toward the protozoan nature of rabies, and that certain protozoan infections are amenable to cure by specific drugs, it is our opinion that research in this direction should not be abandoned. The most promising line of investigation in this connection would appear to be based not upon the physiological action of the drug but upon its action upon the parasite. Here we are limited at once by our lack of knowledge. Administration by the intraspinal method should not be neglected.

Attempts to improve upon the methods of the immunization of exposed persons are desirable, in order to eliminate the small percentage of failures and the very much smaller proportion of injurious results. In making such attempts, however, we must not jeopardize human life by the use of methods which are without experimental basis. The ill effects which have occurred in a very small proportion of treated cases should be traced, if possible, to their source, to determine whether they are really attributable to the treatment, and if so to what element thereof, so that it may be remedied; or whether they really represent a benign influence of the treatment in modifying an otherwise fatal case of rabies into a mild form.

Finally, as regards the eradication of rabies, we have now and have had for a long time all the knowledge of rabies necessary to effect its entire suppression. This knowledge can be summed up in a single sentence, to wit: Rabies is perpetuated in the dog through the infliction of bites by a rabid dog and does not arise spontaneously. If all rabid dogs could be prevented from biting other animals, rabies would in the course of a year be a mere historical curiosity of medicine, an illegitimate field of research for the investigator in pure pathology, a plaything for the controversialist. There are few infectious diseases the prevention of which rests, as in rabies, upon a single definite measure. The public can not say with reference to rabies, "Let the medical profession or scientist show us the cause of this disease and how it may be prevented, and we will do the rest." This information has been furnished, and it is incumbent upon the public, once it has been informed, to bestir itself. Otherwise, the patient work of "patching a bad job" must go on, the slow searching out of better "patches" must continue, and more experimental animals must be sacrificed for the protection of human life.

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LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

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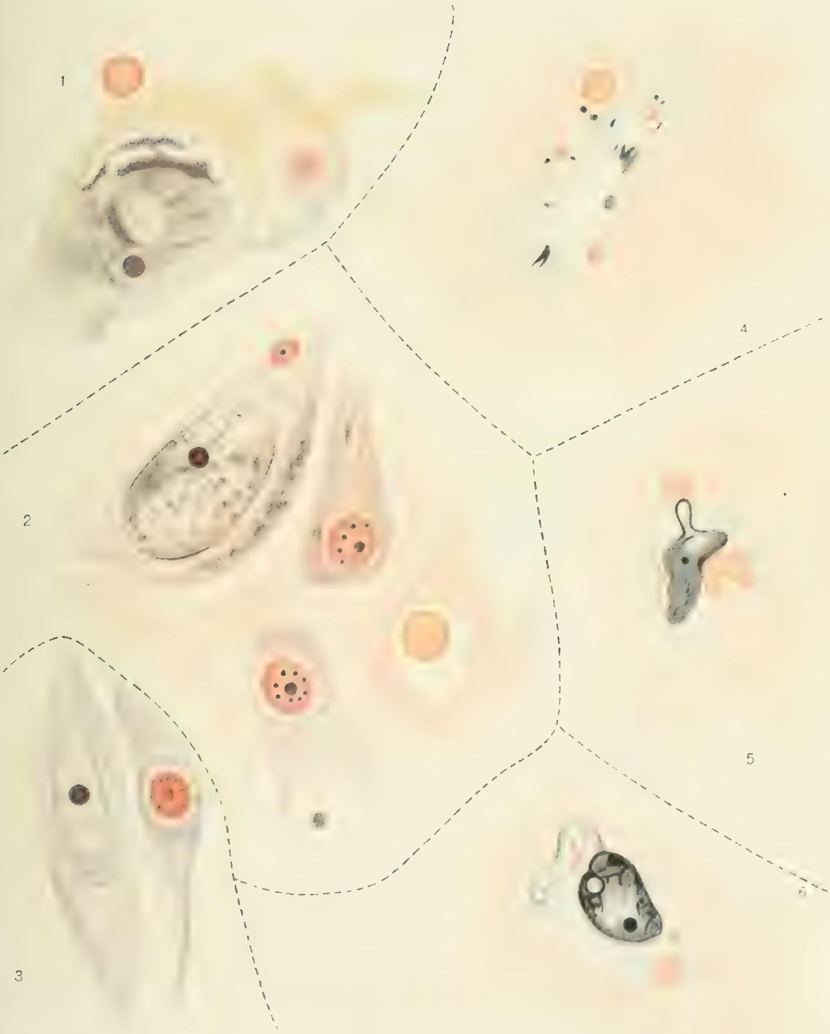
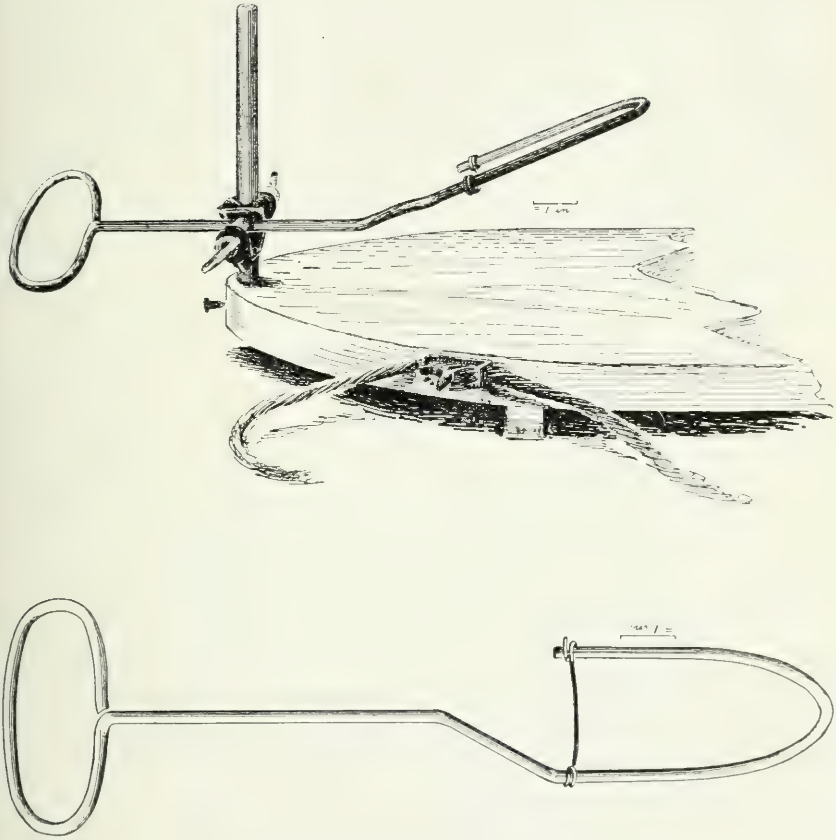
PLATE I.



L. H. Wilder

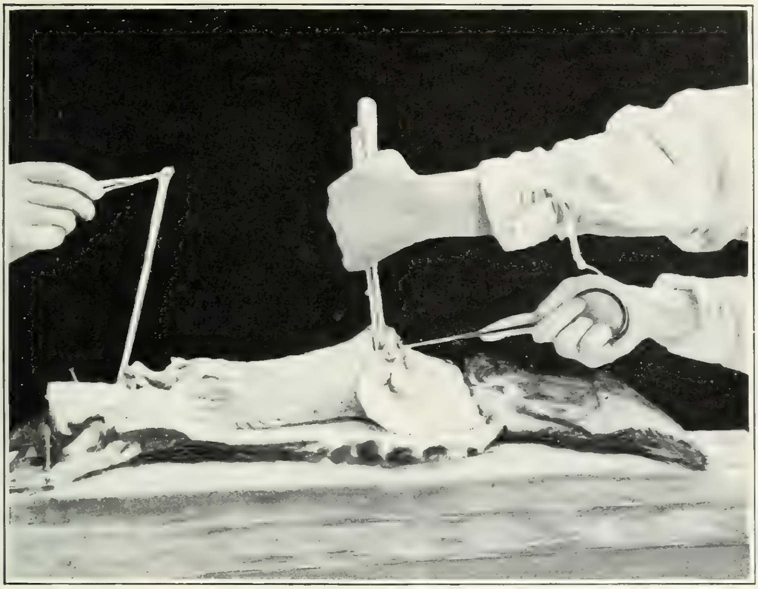
LEGEND.

1, 2, 3,—NEGRI BODIES IN GANGLION CELLS FROM AMMON'S HORN.
4, 5, 6,—BODIES FOUND IN DOG DISTEMPER, AFTER LENTZ.



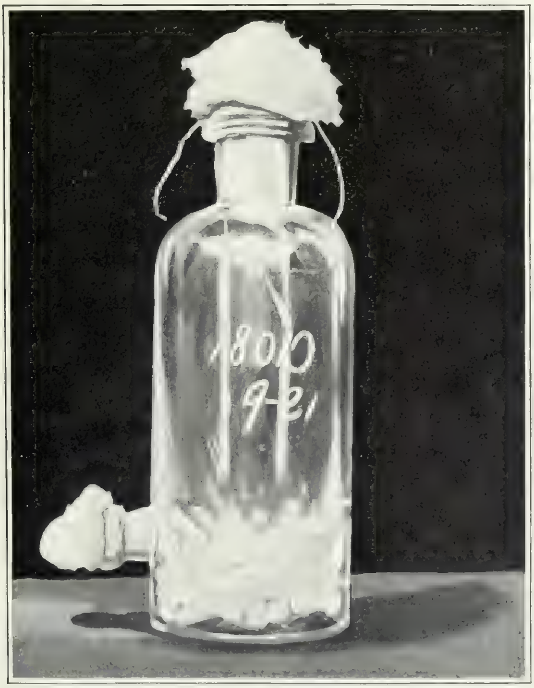
CONVENIENT HOLDER FOR SUBDURAL INOCULATION.

PLATE III.



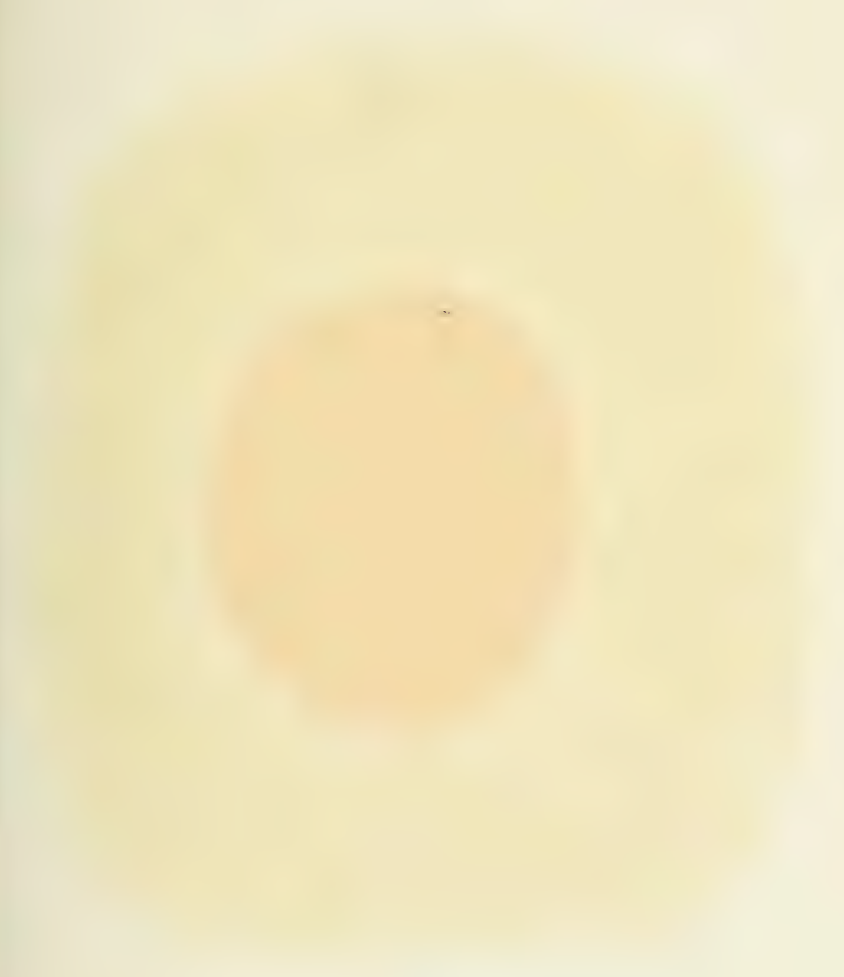
REMOVAL OF THE SPINAL CORD BY THE IMPROVED OSHIDA METHOD.

PLATE IV.



METHOD OF DRYING SPINAL CORDS.

PLATE V.



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LEGEND.

APPEARANCE OF A MODERATELY SEVERE REACTION AT THE SITE OF AN INJECTION.

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Public Health and Marine-Hospital Service of the United States
Walter Wyman, Surgeon-General

HYGIENIC LABORATORY.—BULLETIN No. 66

June, 1910

I. THE INFLUENCE OF AGE AND TEMPERATURE ON THE POTENCY OF DIPHTHERIA ANTITOXIN.—By John F. Anderson

II. AN ORGANISM (*pseudomonas protea*) ISOLATED FROM WATER, AGGLUTINATED BY THE SERUM OF TYPHOID FEVER PATIENTS.—By W. H. Frost

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WASHINGTON
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United States Public Health and Marine-Hospital Service.

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I. THE INFLUENCE OF AGE AND TEMPERATURE ON THE POTENCY OF DIPHTHERIA ANTITOXIN.

By John F. Anderson.

THE INFLUENCE OF AGE AND TEMPERATURE ON THE POTENCY OF DIPHTHERIA ANTITOXIN.[a]

By JOHN F. ANDERSON,
Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service, Washington, D. C.

INTRODUCTION.

When the act of Congress approved July 1, 1902, and the regulations made in accordance therewith providing for the federal supervision of viruses, serums, toxins, and analogous products went into effect one of the first questions raised by the manufacturers was as to the dating of serum. Section 1 of this act required, among other things, that each package of these products should bear a "date beyond which the contents can not be expected beyond reasonable doubt to yield their specific results." At the time of the passage of this law most of the producers dated their diphtheria antitoxin to be returned in nine months; a few made the period as long as one year. At that time there was little known as to the rate of decrease in potency of antitoxin when kept under varying conditions, and there was a demand from the producers for this data for commercial reasons, and from the physicians from the standpoint of therapeutic efficiency, some physicians even doubting the propriety of using antitoxin even in cases of emergency when the return date had expired.

Previous to 1906 there had been some work on the keeping qualities of diphtheria antitoxin reported, but all of it was open to the criticism that the potency had not been determined, either in the beginning or at the end of the experiments, within sufficiently close limits to justify definite conclusions as to the influence of age and temperature.

---

[a] Manuscript submitted for publication October 1, 1909.

In 1906 several of the manufacturers of antitoxin requested the Hygienic Laboratory to take up this question and endeavor to definitely determine the rate of decrease in potency of diphtheria antitoxin, so that they might be able to decide how much excess to allow for this inevitable loss in strength. All of the licensed manufacturers were invited to contribute serum for this purpose, and the following responded, to whom I am indebted for the sera and data in regard to the same: Parke, Davis & Co., Frederick Stearns & Co., Dr. H. M. Alexander & Co., Dr. W. H. Park, New York City department of health.

In all there were 18 different lots of serum furnished, 14 of them being the untreated serum and 4 concentrated by the Gibson process. It is regretted that the number of both kinds of serum was not larger, but it is believed that the number was sufficient to provide data for a definite opinion as to the influence of age and temperature on the potency of diphtheria antitoxin.

PREVIOUS WORK BY OTHERS.

Alba [a] gives the results of retests of 10 lots of diphtheria antitoxin and concludes that antitoxin retains its potency unchanged for a long time. His results are of little value to us, as we know that the method of testing the potency of serums prior to the use of the present method of Ehrlich were not accurate.

Marx [b] gives the results of the retesting of 1,104 samples of serum at the Institut in Frankfort, sent there between March, 1895, and June, 1903. Of this number, only 34, or 3.08 per cent, were withdrawn on account of a decrease in potency. The losses by months were as follows: In three to four months, 2 samples; six to ten months, 9 samples; ten to twenty months, 23 samples. He concludes that the loss in potency, when it does take place, is gradual and that the lack of confidence in old sera is not well founded.

An editorial in the Journal of the Royal Army Medical Corps [c] gives the results of the retesting of six lots of serum that had been for a year either in military hospitals at home or had made one or two voyages to India on transports during that time. Only four of the six lots had lost in potency, the loss varying from 5 to 18 per cent. The conclusion drawn was that antitoxin retains its activity little, if any, impaired at least twelve months.

---

[a] Alba, Fr.: Ueber die Dauer des toxischen und antitoxischen Vermögens beim Diphtherietoxin und Antitoxin. Centblt. f. Bakt., Orig. vol. 23, 1898, p. 934.

[b] Marx, E.: Mitteilungen aus der prufungstechnischen Praxis. Festschrift von Robert Koch, 1903, pp. 451–462.

[c] Diphtheria antitoxin. Editorial. Journ. Roy. Army Med. Corps, vol. 2, 1904, pp. 601–602.

Miller[a] tested the strength of a large number of packages of serum returned to the manufacturers. The time between tests varied from seven months to more than six years. Of 82 lots tested, 30 per cent showed a loss in potency varying from 0 to 50 per cent. It was found that the high potency serum underwent a greater decrease in potency than the low; but that every package, when retested, contained at least the number of units claimed on the label due to the excess allowed by the manufacturer for deterioration.

Layson[b] studied the decrease in potency of diphtheria antitoxin kept under known conditions from one to four years. His first tests were made on 14 lots of serum kept in bulk for one year at 4° C., then kept in a cool room (temperature not stated) for a year, after which they were kept at room temperature for the remainder of the time. The loss on retesting from three years eight months to one year three months later was from 40 to 12.5 per cent.

The second series of tests were on 16 lots of serum in sealed bulbs kept in the ice chest at 4° C. from three years seven months to two years six months. The maximum loss was 16.66 per cent, half of the number showing no loss.

Seven lots that had been on the market and were returned after the expiration of the time limit were tested and found to have lost from 16.7 per cent to no loss in two lots. Of seven lots kept in bulk in the ice chest from ten months to sixteen months, only one showed loss.

Kinyoun and Hitchens[c] tested the loss in strength of 100 lots of serum that had been returned after being on the market and kept under unknown conditions from thirteen to twenty-seven months. They state that in the original tests the unit strength was determined within 50 units, but that on the retest within 10 units. It was found that the loss in unit value ranged from 0 to 48.6 per cent, and that at least 65 per cent of the packages, irrespective of the unit strength, showed a depreciation in value of 25 per cent for each twelve to fifteen months. They conclude that an excess of 24 per cent should be added to all serum on the market to allow for deterioration in potency. This study is of particular value on account of it having been made on a large number of samples kept under commercial conditions.

---

[a] Miller, E. C. L.: On the keeping qualities of antidiphtheria serum. Centblt. f. Bakt. Orig. vol. 38, 1905, pp. 233–236.

[b] Layson, L. C.: Report on a series of experiments to determine the antitoxic depreciation of antidiphtheria serum. Am. Med., vol. 10, 1905, pp. 746–748.

[c] Kinyoun, J. J., and Hitchens, A. P.: On the deterioration of diphtheria antitoxin. Centblt. f. Bakt., Ref., vol. 40, 1907, No. 13.

PLAN AND METHOD OF STUDY.

As early as possible after the serum was received at the laboratory, its potency was determined within 10 units. It was then divided into three lots: One lot was kept at room temperature, one in the cold room at 15° C., and the third in the ice chest at 5° C., all three lots being kept in the dark. The serum was furnished by the contributors either in small vials containing about 3 cubic centimeters each, or in the usual "ready to use" syringe, in which antitoxin is commonly marketed in the United States. In this way an unopened package was used for each successive test. Every six months thereafter the potency of each lot was again determined within 10 units. All tests subsequent to the first were so made that the highest potency tested for was the strength determined at the previous test; in this way there was always shown in each test the decrease in potency since the last test and an approximate indication of the decrease for the next six months. In every instance two guinea pigs were placed on each dose, in order that any irregularities might be checked and avoided. All guinea pigs used weighed between 250 and 280 grams and were from the stock raised at the laboratory and known to have no inherited immunity to diphtheria toxin. At least two, and usually three guinea pigs as controls, were given the L+ dose of toxin used for the test against the standard unit. An effort was always made to place the lower limit of the test so that some of those animals that failed to die an acute death would develop paralysis, and some very interesting data were obtained as to the neutralization of diphtheria toxin by antitoxin.

TABLES SHOWING DECREASE IN POTENCY OF ANTITOXIN AT DIFFERENT TEMPERATURES.

In the following tables will be seen the results of the tests of the eighteen lots of sera in biennial periods when kept at room temperature, 15° C., and 5° C.

The serum kept at room temperature was in the large bacteriological laboratory room; in winter the temperature of this room was about 70° F.; in summer it varied with the diurnal variations of Washington, D. C., which sometimes reached a maximum temperature of 95° to 100° F.

The heading to each table shows the age of the serum when the experiment was begun, potency at that time, and amount and kind of preservative used.

The table shows the interval in days of each test from the beginning of the experiment, the potency at each test, loss in units per cubic centimeter, and per cent loss from original strength.

Tables Nos. 1 to 14 give the results with the untreated antitoxic serum; tables Nos. 15 to 18, with serum precipitated and concentrated by the Gibson process.

TABLE No. 1.

Serum No. 1110; Frederick Stearns & Co.
Blood drawn August 29, 1904; serum preserved with 0.4 per cent trikresol; 549 days from collection of serum to beginning of experiment; strength when experiment was begun, 240 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	181	220	20	8.3
15° C.	....	230	10	4.2
5° C.	184	230	10	4.2
Room.	369	210	30	12.5
15° C.	....	220	20	8.3
5° C.	....	220	20	8.3
Room.	542	200	40	16.7
15° C.	....	220	20	8.3
5° C.	....	220	20	8.3
Room.	722	200	40	16.7
15° C.	....	210	30	12.5
5° C.	....	220	20	8.3
Room.	1,116	150	90	37.5
15° C.	....	200	40	16.7
5° C.	....	210	30	12.5

TABLE No. 2.

Serum No. 08034; Parke, Davis & Co.
Blood drawn September 16, 1905; serum preserved with 0.4 per cent trikresol; 70 days from collection of serum to beginning of experiment; strength when experiment was begun, 310 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	205	290	20	6.4
15° C.	....	310	0	0.0
5° C.	....	310	0	0.0
Room.	374	250	60	19.3
15° C.	....	290	20	6.5
5° C.	....	300	10	3.2
Room.	547	240	70	22.6
15° C.	....	270	40	12.9
5° C.	....	280	30	9.7
Room.	735	230	80	25.8
15° C.	....	250	60	19.4
5° C.	....	280	30	9.7
Room.	1,102	180	130	41.9
15° C.	....	230	80	25.8
5° C.	....	270	40	12.9

TABLE NO. 3.

Serum No. 192; Dr. H. M. Alexander & Co.

Blood drawn August 14, 1905; serum preserved with 0.4 per cent trikresol; 191 days from collection of serum to beginning of experiment; strength when experiment was begun 380 units.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	181	340	40	10.5
15° C.	184	360	20	5.3
5° C.	......	360	20	5.3
Room.	364	330	50	13.2
15° C.	....	340	40	10.5
5° C.	......	330	50	13.2
Room.	540	280	100	26.3
15° C.	....	310	70	18.4
5° C.	....	330	50	13.2
Room.	730	270	110	29.0
15° C.	....	300	80	21.1
5° C.	....	310	70	18.4
Room.	1,113	240	140	36.8
15° C.	....	280	100	26.3
5° C.	....	300	80	21.0

TABLE NO. 4.

Serum No. 08033 Parke Davis & Co.

Blood drawn September 16, 1905; serum preserved with 0.4 per cent trikresol; 80 days from collection of serum to beginning of experiment; strength when experiment was begun, 390 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	191	360	30	7.7
15° C.	193	370	20	5.1
5° C.	......	390	0	0.0
Room.	364	330	60	15.4
15° C.	...	350	40	10.3
5° C.	..	370	20	5.1
Room.	547	300	90	23.0
15° C.		350	40	10.3
5° C.		370	20	5.1
Room.	733	260	130	33.3
15° C.		320	70	17.9
5° C.		340	50	12.8
Room.	1,080	250	140	35.9
15° C.		310	80	20.5
5° C.	....	330	60	15.4

TABLE NO. 5.

Serum No. 08023; Parke Davis & Co.

Blood drawn September 17, 1905; serum preserved with 0.4 per cent trikresol; 151 days from collection of serum to beginning of experiment; strength when experiment was begun, 390 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	180	340	50	12.8
15° C.	181	360	30	7.7
5° C.	....	370	20	5.1
Room.	362	290	100	25.6
15° C.	....	320	70	17.9
5° C.	....	360	30	7.7
Room.	541	280	110	28.2
15° C.	....	310	80	20.5
5° C.	....	360	30	7.7
Room.	718	220	170	43.6
15° C.	....	310	80	20.5
5° C.	....	350	40	10.3
Room.	1,119	160	230	59.0
15° C.	....	300	90	23.0
5° C.	....	330	60	15.4

TABLE NO. 6.

Serum No. 1351; Frederick Stearns & Co.

Blood drawn February 13, 1905; serum preserved with 0.4 per cent trikresol; 385 days from collection of serum to beginning of experiment; strength when experiment was begun, 400 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	181	360	40	10.0
15° C.	184	380	20	5.0
5° C.	....	380	20	5.0
Room.	364	340	60	15.0
15° C.	....	360	40	10.0
5° C.	....	380	20	5.0
Room.	536	320	80	20.0
15° C.	....	350	50	12.5
5° C.	....	360	40	10.0
Room.	722	300	100	25.0
15° C.	....	340	60	15.0
5° C.	....	360	40	10.0
Room.	1,112	240	160	40.0
15° C.	....	310	90	22.5
5° C.	....	350	50	12.5

TABLE No. 7.

Serum No. 08021; Parke Davis & Co.

Blood drawn September 17, 1905; serum preserved with 0.4 per cent trikresol; 101 days from collection of serum to beginning of experiment; strength when experiment was begun, 460 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	183	410	50	10.9
15° C.	....	430	30	6.5
5° C.	....	450	10	2.2
Room.	365	360	100	21.7
15° C.	....	430	30	6.5
5° C.	....	450	10	2.2
Room.	546	340	120	26.1
15° C.	....	420	40	8.7
5° C	....	430	30	6.5
Room.	730	320	140	30.4
15° C.	....	380	80	17.4
5° C.	....	390	70	15.2
Room.	1,068	240	220	47.8
15° C.	....	360	100	21.7
5° C.	....	380	80	17.4

TABLE No. 8.

Serum No. 1429; Frederick Stearns & Co.

Blood drawn May 8, 1905; serum preserved with 0.4 per cent trikresol; 253 days from collection of serum to beginning of experiment; strength when experiment was begun, 520 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	181	480	40	7.7
15° C.	....	480	40	7.7
5° C.	184	490	30	5.8
Room.	365	420	100	19.2
15° C.	....	460	60	11.5
5° C.	....	480	40	7.7
Room.	538	380	140	26.9
15° C.	....	440	80	15.4
5° C.	....	480	40	7.7
Room.	730	360	160	30.8
15° C.	....	420	100	19.2
5° C.	....	460	60	11.5
Room.	1,113	340	180	34.6
15° C.	....	400	120	23.1
5° C.	....	420	100	19.2

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TABLE No. 9.

Serum No. 07635; Parke Davis & Co.

Blood drawn May 1, 1905; serum preserved with 0.4 per cent trikresol; 240 days from collection of serum to beginning of experiment; strength when experiment was begun, 660 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	183	580	80	12.1
15° C.		600	60	9.1
5° C.		620	40	6.1
Room.	366	500	160	24.2
15° C.		580	80	12.1
5° C.		620	40	6.1
Room.	544	440	220	33.3
15° C.		500	160	24.2
5° C.		580	80	12.1
Room.	742	340	320	48.5
15° C.	730	500	160	24.2
5° C.		540	120	18.2
Room.	1,089	300	360	54.5
15° C.		420	240	36.4
5° C.		500	160	24.2

TABLE No. 10.

Serum No. 08022; Parke Davis & Co.

Blood drawn September 17, 1905; serum preserved with 0.4 per cent trikresol; 100 days from collection of serum to beginning of experiment; strength when experiment was begun, 670 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	180	580	90	13.4
15° C.		640	30	4.5
5° C.		640	30	4.5
Room.	365	560	110	16.4
15° C.		620	50	7.5
5° C.		640	30	4.5
Room.	539	480	190	28.4
15° C.		600	70	10.5
5° C.		620	50	7.5
Room.	718	420	250	37.3
15° C.		540	130	19.4
5° C.		600	70	10.5
Room.	1,119	300	370	55.2
15° C.		500	170	25.4
5° C.		560	110	16.4

TABLE NO. 11.

Serum No. 1500; Frederick Stearns & Co.

Blood drawn August 21, 1905; serum preserved with 0.4 per cent trikresol; 374 days from collection of serum to beginning of experiment; strength when experiment was begun, 1,100 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	180	1,040	60	5.5
15° C.	....	1,060	40	3.6
5° C.	....	1,060	40	3.6
Room.	365	880	220	20.0
15° C.	380	960	140	12.7
5° C.	....	1,020	80	7.3
Room.	545	840	260	23.6
15° C.	....	940	160	14.5
5° C.	....	1,000	100	9.1
Room.	748	800	300	27.5
15° C.	....	900	200	18.2
5° C.	....	960	140	12.7
Room.	928	700	400	36.4
15° C.	....	860	240	21.8
5° C.	....	940	160	14.5
Room.	1,082	660	540	49.1
15° C.	....	840	260	23.6
5° C.	....	940	160	14.5

TABLE NO. 12.

Serum No. 305; New York health department.

Blood drawn May 18, 1906; serum preserved with chloroform; 20 days from collection of serum to beginning of experiment; strength when experiment was begun, 1,190 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	183	1,100	90	7.6
15° C.	....	1,190	0	0.0
5° C.	....	1,190	0	0.0
Room.	366	980	210	17.6
15° C.	....	1,080	110	9.2
5° C.	369	1,120	70	5.9
Room.	539	860	330	27.7
15° C.	....	1,040	150	12.6
5° C.	....	1,120	70	5.9
Room.	733	800	390	32.8
15° C.	....	1,000	190	16.0
5° C.	....	1,100	90	7.6
Room.	928	780	410	34.5
15° C.	....	980	210	17.6
5° C.	....	1,100	90	7.6
Room.	1,095	760	430	36.1
15° C.	....	960	230	19.3
5° C.	....	1,020	170	14.3

TABLE No. 13.

Serum No 306; New York health department
Blood drawn May 18, 1906; serum preserved with chloroform; 20 days from collection of serum to beginning of experiment; strength when experiment was begun, 1,400 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	194	1,120	280	20.0
15° C.	....	1,380	20	1.4
5° C.	....	1,400	0	0.0
Room.	369	1,100	300	21.4
15° C.	....	1,280	120	8.6
5° C.	371	1,280	120	8.6
Room.	545	900	500	35.7
15° C.	....	1,220	180	12.9
5° C.	....	1,260	140	10.0
Room.	735	740	660	47.1
15° C.	....	1,120	280	20.0
5° C.	....	1,200	200	14.3
Room.	924	680	720	51.4
15° C.	....	1,000	400	28.6
5° C.	....	1,160	240	17.1
Room.	1,095	660	740	52.9
15° C.	....	960	440	31.6
5° C.	....	1,140	260	18.6

TABLE No. 14.

Serum No. 310; New York health department.
Blood drawn June 4, 1906; serum preserved with chloroform; 88 days from collection of serum to beginning of experiment; strength when experiment was begun, 1,500 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
5° C.	180	1,440	60	4.0
Room.	379	1,180	320	21.3
15° C.	364	1,340	160	10.6
5° C.	....	1,360	140	9.3
Room.	562	1,100	400	26.7
15° C.	....	1,300	200	13.3
5° C.	....	1,320	180	12.0
Room.	747	1,020	480	31.8
15° C.	....	1,220	280	18.7
5° C.	....	1,300	200	13.3
Room.	929	980	520	34.7
15° C.	....	1,200	300	20.0
5° C.	....	1,280	220	14.7
Room.	1,080	940	560	37.3
15° C.	....	1,120	380	25.3
5° C.	....	1,280	220	14.7

Table No. 15.

Serum No. 245; Dr. H. M. Alexander Company.

Blood drawn ——; concentrated serum after Gibson's method; concentration completed November 30, 1906; preserved with chloroform; 35 days from concentration of serum to beginning of experiment; strength when experiment was begun, 440 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	179	360	80	18.2
15° C.		400	40	9.1
5° C.		420	20	4.6
Room.	361	300	140	31.8
15° C.		360	80	18.2
5° C.		380	60	13.6
Room.	543	280	160	36.4
15° C.		340	100	22.7
5° C.		360	80	18.2
Room.	751	260	180	40.9
15° C.		300	140	31.8
5° C.		360	80	18.2
Room.	898	250	190	43.2
15° C.		300	140	31.8
5° C.		350	90	20.5

Table No. 16.

Serum No. 10B; New York health department.

Blood drawn ———–; concentrated serum after Gibson's method; concentration completed March 2, 1909; preserved with chloroform; 22 days from concentration of serum to beginning of experiment; strength when experiment was begun, 580 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	178	560	20	3.5
15° C.		540	40	6.9
5° C.		580	0	0.0
Room.	363	520	60	10.4
15° C.		540	40	6.9
5° C.		520	60	10.4
Room.	535	480	100	17.2
15° C.		520	60	10.4
5° C.		520	60	10.4
Room.	761	440	140	24.1
15° C.		490	90	15.5
5° C.		500	80	13.8
Room.	1,101	400	180	31.0
15° C.		480	100	17.2
5° C.		500	80	13.8

TABLE No. 17.

Serum No. 13C; New York health department.

Blood drawn ——; concentrated serum after Gibson's method; concentration completed February 26, 1909; preserved with chloroform; 26 days from concentration of serum to beginning of experiment; strength when experiment was begun, 700 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	184	620	80	11.4
15°C.	....	620	80	11.4
5°C.	....	660	40	5.7
Room.	368	600	100	14.3
15°C.	....	620	80	11.4
5°C.	....	640	60	8.6
Room.	537	580	120	17.1
15° C.	....	600	100	14.3
5° C.	....	640	60	8.6
Room.	761	560	140	20.0
15° C.	....	580	120	17.1
5° C.	....	600	100	14.3
Room.	1,101	520	180	25.7
15° C.	....	580	120	17.1
5° C.	....	600	100	14.3

TABLE No. 18.

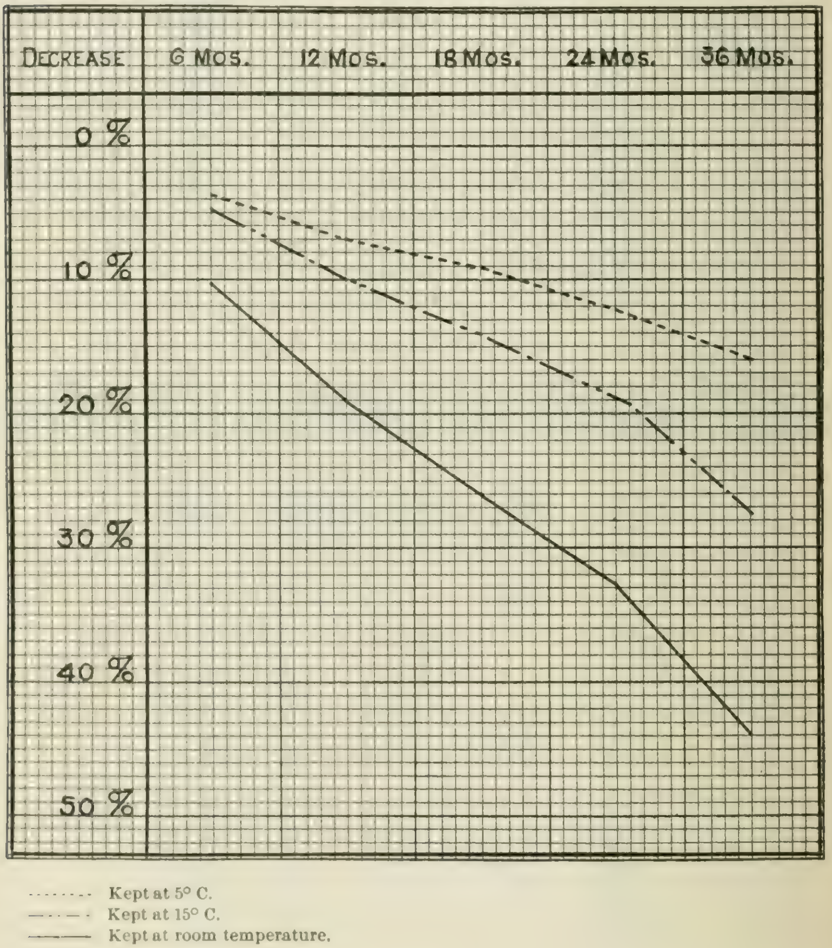
Serum No. A249; Dr. H. M. Alexander Company.

Blood drawn ——; concentrated serum after Gibson's method; concentration completed December 29, 1906; preserved with chloroform; 17 days from concentration of serum to beginning of experiment; strength when experiment was begun, 740 units per cubic centimeter.

Temperature at which serum was kept.	Interval from beginning of experiment.	Strength in units per cubic centimeter.	Loss in units per cubic centimeter.	Loss.
	Days.			*Per cent.*
Room.	182	700	40	5.4
15° C.	....	720	20	2.7
5° C.	....	740	0	0.0
Room.	364	670	70	9.1
15° C.	....	700	40	5.4
5° C.	....	720	20	2.7
Room.	542	670	70	9.1
15° C.	....	660	80	10.8
5° C.	....	700	40	5.4
Room.	750	600	140	18.1
15° C.	....	660	80	10.8
5° C.	....	700	40	5.4
Room.	887	580	160	21.6
15° C.	....	640	100	13.5
5° C.	....	700	40	5.4

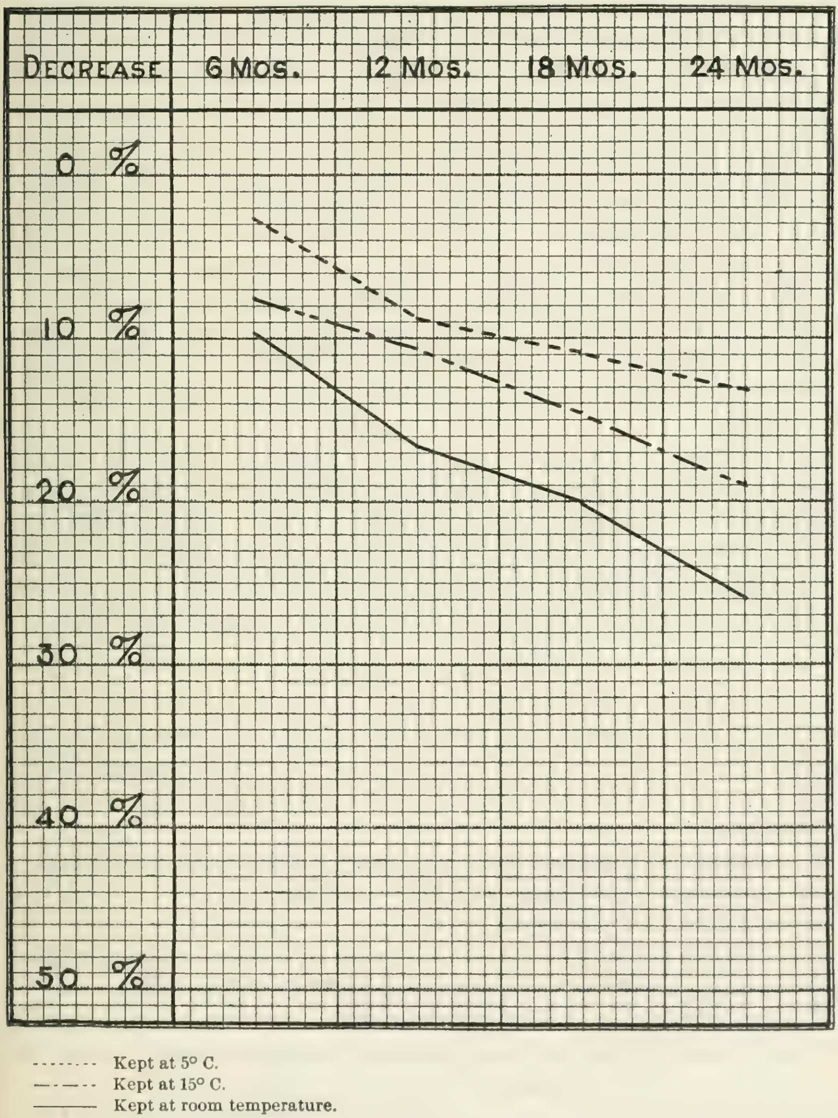
UNTREATED SERUM.

Curves showing average decrease in potency of fourteen lots of untreated serum in six months' intervals when kept at different temperatures.



CONCENTRATED SERUM.

Curves showing average decrease in potency of four lots of concentrated serum in six months' intervals when kept at different temperatures



THE KEEPING QUALITIES OF DRIED ANTITOXIN.

It has been shown by others as well as by me that diphtheria antitoxin in the liquid serum inevitably loses in potency by age, but there does not seem to have been any work reported as to the keeping qualities of antitoxin in the dried state. In May, 1904, I prepared a large amount of dried diphtheria antitoxin to be used in the preparation of the American standard diphtheria unit. Most of this serum was kept in the special bulb that I designed for this purpose,[a] under vacuum and P_2O_5. A few bulbs were kept without the acid and not under vacuum. The dried serum, at the time of its preparation in 1904, contained 5,250 units per gram. Recently I tested the potency of some of the serum kept since 1904 at 5° C., but not under vacuum and P_2O_5, and found it still to contain 5,250 units per gram; in other words, the dried serum in five and one-half years is of the same potency as when first prepared. It is to be regretted that some of the same serum was not placed at room temperature and at 15° C. in order to determine if it retains its potency as well under these conditions as at 5° C. I am inclined to believe that such would have been found to be the case, or at most, the loss would have been very slight.

As the dried serum apparently retains its potency with but slight impairment, it would seem that dried diphtheria antitoxin is peculiarly suited for shipment to the Tropics, or for use on ships that make long voyages. The only drawback to its use appears to be in the making of a sterile solution, and this could be largely overcome by aseptic precautions by the user and the producer of the serum.

SUMMARY AND DISCUSSION.

A study of the preceding tables shows that the average loss in percentage strength of the 14 untreated sera for three years at room temperature was 44.2 per cent; at 15° C., 24.6 per cent; at 5° C., 15.9 per cent.

The average percentage loss in potency of the same sera for two years at room temperature was 32.8 per cent; at 15° C., 18.5 per cent; at 5° C., 12.4 per cent.

The average percentage loss for one year at room temperature was 18.7 per cent; at 15° C., 10.2 per cent; at 5° C., 6.7 per cent.

The average loss in percentage strength of the 4 sera concentrated by the Gibson method for two years at room temperature was 25.8 per cent; at 15° C., 18.8 per cent; at 5° C., 12.9 per cent.

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[a] The immunity unit for standardizing diphtheria antitoxin. Bull. 21, Hygienic Laboratory, Feb., 1905. By M. J. Rosenau.

The average percentage loss of the same sera for one year at room temperature was 16.4 per cent; at 15° C., 10.5 per cent; at 5° C., 8.8 per cent.

From the above, it will be seen that there was but little difference in the keeping qualities of the untreated serum and that concentrated by the Gibson process, except for the serum kept at room temperature for two years, in which case there was a difference in favor of the Gibson serum of about 8 per cent. It would seem that the yearly loss in potency is about 20 per cent, although occasionally it may go as high as 25 per cent when the serum is kept at room temperature. When kept at 15° C., the average yearly loss is about 10 per cent, although it may sometimes be double that, but this is exceptional. At 5° C., the average yearly loss falls to about 6 per cent, but there may be instances in which the loss is considerably greater.

The maximum loss of any serum in three years at room temperature was 59 per cent; at 15° C., 36.4 per cent; at 5° C., 24 per cent; the last two being in the same serum.

The minimum loss for three years at room temperature was 34.6 per cent; at 15° C., 16.7 per cent; and at 5° C., 12.5 per cent; the last two being in the same serum.

One of the very interesting points that was brought out by the experiments was as to the relation of the antitoxic content of a serum to its therapeutic value. It was found that in the test mixtures the protective value of the serum was in exact accord with its unit value; for example, $\frac{1}{1500}$ c.c. of serum No. 310 was of the same protective value as $\frac{1}{150}$ cubic centimeter of serum No. 1110; the first serum contained 1,500 units per cubic centimeter and the last only 150 per cubic centimeter. There can be no question as to the protective value of the serum being in direct relation to its unit value for, in over 200 determinations of the antitoxic potency of serum in this study, the same result was had in each instance.

It was noticed that all sera, even of the same age and kept under the same conditions, did not deteriorate in the same ratio; just why this should be so I am unable to decide, but it is apparently independent of external influences and probably depends on some inherent property of the serum.

The addition of preservatives, such as chloroform and tricresol, apparently did not exert any influence upon the deterioration of the antitoxin as there was no appreciable difference in the behavior of the sera preserved with these two substances.

The opinion held by some physicians that old sera or sera on which the return dates have expired should not be used is without scientific basis, and the only reason for not using such sera would be that a larger amount would have to be given to make up for the decrease in potency. Old sera, unit for unit, is just as potent as fresh sera, and would be, perhaps, less apt to cause severe serum reactions than fresh sera.

CONCLUSIONS.

The average yearly loss in potency of diphtheria antitoxin at room temperature is about 20 per cent; at 15° C., about 10 per cent; at 5° C., about 6 per cent, although in some instances these percentages may be much increased.

From this work, there appears to be but little difference in the keeping qualities of untreated sera and sera concentrated by the Gibson process.

Diphtheria antitoxin to be placed upon the market and there kept under unknown conditions as regards temperature should not be labeled with a return date longer than two years and should contain an excess of at least 33 per cent to allow for decrease in potency; in addition, when the serum is sold in syringes with an absorbable piston, an excess should be added for this loss.

Dried diphtheria antitoxin kept in the dark, at 5° C., retains its potency practically unimpaired for at least five and one-half years.

The lack of confidence in the therapeutic properties of old sera is without basis as such sera, unit for unit, are as potent as new sera.

The protective value of diphtheria antitoxin is in exact accord with its unit value and is independent of the volume of the serum or other properties in the serum.

II. AN ORGANISM (*pseudomonas protea*) ISOLATED FROM WATER, AGGLUTINATED BY THE SERUM OF TYPHOID FEVER PATIENTS.

By W. H. Frost.

II. AN ORGANISM (PSEUDOMONAS PROTEA) ISOLATED FROM WATER, AGGLUTINATED BY THE SERUM OF TYPHOID FEVER PATIENTS.[a]

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INTRODUCTION.

For the last four summers, in connection with the work of the board appointed by the Surgeon-General of the United States Public Health and Marine-Hospital Service to investigate the origin and prevalence of typhoid fever in the District of Columbia, routine bacteriologic examinations have been made in the Hygienic Laboratory of the raw and filtered Potomac River water. During the summer of 1909 this included daily routine bacteriologic examinations of samples of water as follows: (1) From the Potomac River water as it entered the first storage reservoir (either Dalecarlia or Georgetown); (2) from the outlet of the Washington City reservoir, representing the water after short storage and sedimentation, and as applied to the filter beds; (3) the effluent from the filtration plant (storage basin); (4) from a constantly running tap in the city.[b] In addition to these an attempt has been made during the summer and fall of 1909 to derive further information as to the quality of these samples of water by methods not included in the "Standard Methods of Water Analysis." (1)

Following the report by Jackson and Melia (2) on their successful use of lactose bile in isolating the Bacillus typhosus from water, a systematic search has been made for this organism in the samples of water examined, using a modification of the technic described by them. The modification in the technic employed consists in the use of Endo's medium (3) instead of Hesse's agar for plating out from the

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[a] Manuscript received for publication February 1, 1910.

[b] For details of the bacteriological examinations of these waters, see Bull. Hyg. Lab., U. S. Pub. Health and Mar. Hosp. Service, Nos. 35 (1906), 44 (1907), 52 (1908).

lactose bile tubes, a modification adopted because this medium has proved most satisfactory in the Hygienic Laboratory for the isolation of the *Bacillus typhosus*.

From each sample of water examined, 10 c. c. were planted in a lactose bile fermentation tube, and such of these tubes as showed gas formation were plated out on Endo's medium at the end of forty-eight or seventy-two hours. Quite frequently, also, ordinary lactose bouillon tubes showing gas formation were plated out on the same medium. If colonies resembling typhoid colonies appeared on these plates within twenty-four hours, from 5 to 10 such colonies were fished and planted in tubes of ordinary bouillon. Each tube was then tested after twenty-four hours' incubation, with strong typhoid agglutinating serum—one drop from a Pasteur pipette. This makes a serum dilution varying from about 1 : 150 to 1 : 350, in which dilution the typhoid bacillus is readily agglutinated by the serum used in this work—a horse serum of titer 1 : 20,000 for the Hygienic Laboratory stock culture of typhoid. Agglutinated tubes were replated to obtain a pure culture, determined culturally, and studied more accurately in regard to agglutinating properties.

The results of this work have been entirely negative so far as the isolation of the typhoid bacillus is concerned, but the procedure led to the discovery in the filtered water of a pseudomonas which resembles *B. typhosus* in its growth on Endo's medium and which agglutinates quite constantly in comparatively high dilution with antityphoid serum, but which culturally differs widely from *B. typhosus*.

The organism here described was first isolated by the above technic from a lactose bouillon fermentation tube containing 10 c. c. of water collected from a tap in Lafayette Square on August 7, 1909. Although this tube showed gas formation, no *Bacillus coli* or other lactose-fermenting organism appeared on the Endo plates, which showed, however, a number of clear colonies resembling typhoid. Six of these colonies were fished into bouillon and incubated for twenty-four hours, to be tested as usual with typhoid agglutinating serum. On the addition of the serum to the bouillon cultures the agglutination in one tube was so prompt and so marked as to lead to the presumption that the culture was the typhoid bacillus, as the same serum had been used in the laboratory in the examination of several hundred specimens of feces, urine, and blood, and had never been known to agglutinate so characteristically in this dilution any organism except the *Bacillus typhosus*. This suspicion was strengthened by the morphology of the organism and the appearance of the Endo plates made from the agglutinated tube. But the usual routine cultural examination proved that such was not the case.

CHARACTERISTICS.

When obtained in assuredly pure culture by repeated plating, the agglutinating organism showed the following morphologic and cultural characteristics:

Morphology.—Straight rods, single or in short chains, varying from 1.2 to 2.5 μ in length; average length about 1.7 μ; average thickness about 0.6 μ; flagella always polar, usually a single flagellum from only one pole; forms with one flagellum at each pole, or with two flagella at one pole, are relatively rare, and probably represent two or more bacteria in close approximation.

Motility.—Young agar or bouillon cultures are actively motile; motility, however, is sometimes lost in old cultures, or in bouillon cultures frequently transplanted and kept at 37° C.

Spores.—Not observed.

Staining.—Stained readily and uniformly with watery anilin stains; decolorized by Gram's method.

Agar slant.—Growth fairly abundant in twenty-four hours at 37° C.; slightly brownish-white, smooth, moist, soft, edges uniform, not spreading; growth becomes brownish after several days.

Plate colonies.—Agar: Not distinctive; small, round, white, at end of twenty-four hours.

Hesse's agar: Colonies white, wide-spreading, edges becoming radiate.

Lactose-litmus agar: Clear, colorless, transparent, generally slightly larger and less clear than colonies of *B. typhosus.*

Endo's medium: Clear, transparent, colorless, or slightly pinkish; generally larger and less clear than colonies of *B. typhosus.*

Gelatin (12.5 per cent): In twenty-four hours, very small, white; in forty-eight hours, 2 to 5 mm. in diameter, liquefying, round, cup-shaped, grayish with white center; older cultures often present a varying proportion of very slowly liquefying colonies.

On none of the above media are the colonies at all distinctive. On agar, lactose-litmus agar, and Endo's medium they may easily be mistaken for typhoid colonies.

Glycerin potato: Growth in twenty-four hours at 37° C., visible but scant; white, moist; becomes brownish after several days, but remains scant.

Gelatin stab: At 37° C., liquefied in twenty-four hours; at 20° C., liquefaction always takes place, forming a deep funnel-shaped depression; the rapidity of the liquefaction, however, varies greatly in different strains, and in the same strain at different times.

Blood serum: Liquefied with varying rapidity; usually noticeable in one to four days at 37° C.

Bouillon: Uniform turbidity in twenty-four hours; reaction alkaline after a few days.

Litmus milk: Slight acidity in twenty-four hours, with formation of a semisolid clot; solution of the casein, complete or partial, at a very inconstant rate; terminal reaction (ten to twenty days) usually acid, sometimes alkaline. The initial acidity and coagulation of litmus milk are constant characteristics. Solution of the casein is a very inconstant characteristic. All strains when freshly isolated peptonized milk rapidly; after several months on artificial culture media this property is diminished in all strains, and varies greatly with different lots of litmus milk. The production of alkali is also inconstant. As nearly as can be ascertained, the production of a high degree of primary acidity in litmus milk retards or prevents peptonization; and subsequent alkali formation, if taking place at all in such cases, is insufficient to neutralize the acid already formed.

Fermentation tube: Dextrose bouillon, fermented with production of acid and 10 to 30 per cent gas in twenty-four hours; saccharose bouillon, neither acid nor gas; lactose bouillon, neither acid nor gas; growth in closed arm in all the above media.

Peptone solution (1 per cent): Indol reaction very marked in four days.

Nitrate bouillon: Nitrites and ammonia formed.

Odor.—Disagreeable, putrefactive.

Optimum temperature.—Growth most rapid at 37° C. Grows readily but less rapidly at 20° C.

A correlation of this organism with previously described species is difficult, chiefly because so many of the descriptions given are not sufficiently exclusive. Especially is this true as regards morphology. According to the systems of Migula (4) and of Chester (5), the primary basis of classification is morphologic. This organism, according to their systems, must be classed as a pseudomonas, and can not be placed in a group with bacilli. Biologically, however, it is closely related to the Proteus group of bacilli, as described by Fuller and Johnson (6), Ford (7), and Jordan (8). The most recent of these writers, Jordan (8), in defining the Proteus group, says:

> The members of this group ferment dextrose and sucrose, rarely lactose. They are, for the most part, vigorously proteolytic, rapidly liquefying gelatin and blood serum, and precipitating and then dissolving casein.

Ford's Proteus group includes only bacilli which render milk acid, with subsequent production of alkali, and peptonize with or without coagulation. He differentiates three species in this group by their fermentation of sugars—one fermenting dextrose, saccharose, and lactose; one fermenting dextrose and lactose; and one fermenting dextrose and saccharose.

None of these authors has described the flagella of these organisms, so it is possible that their Proteus groups may contain some pseudomonas, although *Bacillus vulgaris* (Hauser) Migula (=*proteus vulgaris*, Hauser), is a true bacillus with numerous peritrichic flagella.

The organism derived from the Washington filtered water is differentiated from the "Proteus group" of bacilli primarily by its morphology, being a pseudomonas; also by its failure to ferment either saccharose or lactose.

The various species of pseudomonas described by Migula and Chester are so imperfectly described that it is impossible from their descriptions to make accurate differentiations. The organisms whose descriptions seem most closely to conform to this organism are *Ps. liquefaciens*, (Tataroff) Migula, and *Ps. liquida*, (Frankland) Chester. The very broad descriptions of these organisms, however, make it seem probable that they represent a whole group, within which a number of species can be differentiated by modern methods.

The organism here described is considered to properly belong in a group of pseudomonas, characterized by growth at 37° C., nonproduction of pigment, liquefaction of gelatin, casein, and blood serum, and probably showing various fermentations of carbohydrates.

By a brief study of about 20 colonies isolated from various waters by various methods, I have been able to isolate two varieties of pseudomonas identical with the above-described pseudomonas morphologically and biologically except in their action on carbohydrates, so that we have three varieties of pseudomonas, otherwise identical, which are distinguished as follows:

Fermentation of carbohydrates.

	Dextrose.		Saccharose.		Lactose.	
	Acid.	Gas.	Acid.	Gas.	Acid.	Gas.
1. Species above described	+	+	−	−	−	−
2. Species No. 2	+	+	+	+	−	−
3. Species No. 3	+	−	−	−	−	−

Any one of these three varieties answers equally well to Migula's description of *Ps. liquefaciens* (Tataroff), and to Chester's description of *Ps. liquida* (Frankland). For the sake of clearness, then, the pseudomonas which I have described is provisionally classed as a new species, *Pseudomonas protea*, so called by reason of its biologic resemblance to the proteus group of bacilli, as well as the variability of some of its properties.

IDENTITY OF THE VARIOUS STRAINS ISOLATED.

Nine strains of this organism, isolated from as many different samples of water on various dates, have been studied and found identical by the above criteria, as well as by specific agglutination tests. Variations have been noted from time to time in their proteolytic properties, but these variations have not been sufficiently constant to differentiate one strain from another. Two strains of pseudomonas, isolated by a different technic and completely identified with *Ps. protea*, except in agglutinating properties, are more fully described below. (See Tables 13 and 14.)

PATHOGENICITY.

The pathogenicity of *Ps. protea* has not been studied in detail. One-half cubic centimeter of a twenty-four-hour bouillon culture of a freshly isolated strain (No. 366), injected intraperitoneally into a guinea pig of 225 grams weight, caused death in thirty hours. Autopsy showed a general acute fibrinous peritonitis. The organism was recovered in pure culture from the peritoneal cavity and the heart.

Subsequently, 0.1 c. c. and 0.5 c. c., respectively, of another strain (No. 368), injected into guinea pigs of 365 and 355 grams, caused illness of moderate severity, of twenty-four hours and forty-eight hours duration. This strain had been cultivated on artificial media for several months.

A third strain (No. 370) after more than four months on artificial culture media, killed one out of two guinea pigs injected intraperitoneally with 0.5 c. c. of a twenty-four-hour bouillon culture, while a third pig injected with 1.0 c. c. of the same culture survived.

The sterile filtrate of twenty-four and forty-eight hour bouillon cultures of several strains, obtained by filtering through a Pasteur-Chamberland filter (F) has proven toxic and occasionally fatal to guinea pigs of about 300 grams, in doses of 2 cubic centimeters, intraperitoneally. The toxic effects produced were more or less intense enteritis and peritonitis. In one case a prolapse of the rectum occurred.

PREVALENCE IN RAW AND FILTERED WATER.

Following the isolation of the first strains of this organism, an effort was made to determine its prevalence in the unfiltered and filtered water, attention being especially directed to the latter, as very few samples of the filtered water had shown gas formation in fermentation tubes, and consequently very few had, up to this time, been examined by this technic. From this time forward, as many tubes as possible were plated out on Endo's medium, whether showing gas formation or not. The following table shows the number, dates, and results of the examinations of samples of the filtered water:

Table No. 1.—*Samples of filtered water (storage and tap) examined for the presence of Pseudomonas protea.*

Laboratory No. of sample.	Date.	Source.	Amount water planted.	Fermentation tubes.		B. coli.	Pseudomonas protea.
				Medium.	Gas, 48 hours.		
			c. c.		*Per cent.*		
362^{t}	Aug. 7, 1909	Tap	10	Lactose bouillon	24	—	+ (362^{t}).
366	Aug. 10, 1909	Storage	10	Bouillon	14	—	—
366	do	do	10	Lactose bile	0	—	+ (366).
366^{t}	do	Tap	10	Bouillon	32	-	
366^{t}	do	do	10	Bile	0	—	+ (366^{t}).
368	Aug. 11, 1909	Storage	10	Bouillon	0	—	—
368	do	do	10	Bile	0	—	+ (368).
368^{t}	do	Tap	10	Bouillon	0	—	—
368^{t}	do	do	10	Bile	0		—
370	Aug. 12, 1909	Storage	10	Bouillon	0	—	+ (370).
370	do	do	10	Bile	0	—	—
370^{t}	do	Tap	10	Bouillon	0	—	—
370^{t}	do	do	10	Bile	0	—	+ (370^{t}).
372	Aug. 13, 1909	Storage	10	Bouillon	0	—	—
372	do	do	10	Bile	0	—	—
372^{t}	do	Tap	10	Bouillon	10	—	+ (372^{t}).
372^{t}	do	do	10	Bile	0	—	-
374	Aug. 14, 1909	Storage	10	Bouillon	0	—	—
374	do	do	10	Bile	0	—	—
374^{t}	do	Tap	10	Bouillon	15	—	—
374^{t}	do	do	10	Bile	0	—	+ (374^{t}).
422	Sept. 13, 1909	Storage	10	Bouillon	0	—	-
422	do	do	10	Bile	0	—	—
422t	do	Tap	10	Bouillon	0	—	—
422^{t}	do	do	10	Bile	0	—	—
424	Sept. 14, 1909	Storage	10	do	0	—	+? (culture lost).
424^{t}	do	Tap	10	do	0	—	-
434	Sept. 20, 1909	Storage	10	do	0	—	—
434^{t}	do	Tap	10	do	0	—	—[b]
460	Oct. 5, 1909	Storage	10	Bouillon	10	—	-
460	do	do	10	Bile	0	—	—
460^{t}	do	Tap	10	Bouillon	0	—	—
460^{t}	do	do	10	Bile	0	—	—
468	Oct. 9, 1909	Storage	10	Bouillon	22	—	—[b]
468	do	do	10	Bile	0	—	+ (468^{b}).
468^{t}	do	Tap	10	Bouillon	0	—	—
470	Oct. 11, 1909	Storage	10	do	0	—	—
470^{t}	do	Tap	10	Bile	0	—	—
472	Oct. 12, 1909	Storage	10	Bouillon	0	—	—
472	do	do	10	Bile	0	—	—
472^{t}	do	Tap	10	Bouillon	0	—	—
472^{t}	do	do	10	Bile	0	—	—
504	Oct. 30, 1909	Storage	10	do	0	—	—
504^{t}	do	Tap	10	do	0	—	—
506	Nov. 1, 1909	Storage	10	Bouillon	11	—	—
506	do	do	10	Bile	0	-	—
506^{t}	do	Tap	10	Bouillon	0	—	—
506^{t}	do	do	10	Bile	0	—	—
508^{t}	Nov. 2, 1909	do	10	Bouillon	12	-	—
510	Nov. 3, 1909	Storage	10	Bile	0	—	—
510^{t}	do	Tap	10	do	0	—	-
516	Nov. 6, 1909	Storage	10	Bouillon	0	—	—
516	do	do	10	Bile	0	—	—
516^{t}	do	Tap	10	Bouillon	0	—	—
516^{t}	do	do	10	Bile	......	—	—
518	Nov. 8, 1909	Storage	10	Bouillon	0	—	—
518	do	do	10	Bile	0	—	-
518^{t}	do	Tap	10	Bouillon	0	—	—
518^{t}	do	do	10	Bile	0	—	—
528	Nov. 13, 1909	Storage	10	Bouillon	0	—	—
528	do	do	10	Bile	0	—	—
528^{t}	do	Tap	10	Bouillon	0	—	—
528^{t}	do	do	10	Bile	0	—	—
530	Nov. 15, 1909	Storage	10	Bouillon	0	-	—
530	do	do	10	Bile	0	—	—
530^{t}	do	Tap	10	Bouillon	5	—	—
530^{t}	do	do	10	Bile	0	—	—
540	Nov. 20, 1909	Storage	10	Bouillon	0	-	—
540	do	do	10	Bile	0	-	—
540^{t}	do	Tap	10	Boulilon	0	-	—
540^{t}	do	do	10	Bile	0	—	—

TABLE No. 1.—*Samples of filtered water (storage and tap) examined for the presence of Pseudomonas protea*—Continued.

Laboratory No. of sample.	Date.	Source.	Amount water planted.	Fermentation tubes.		B. coli.	Pseudomonas protea.
				Medium.	Gas, 48 hours.		
			c. c.		*Per cent.*		
544	Nov. 23, 1909	Storage	10	Bouillon	10	–	–
544^t	do	Tap	10	Bile	0	–	–
550	Nov. 27, 1909	Storage	10	Bouillon	0	–	–
550	do	do	10	Bile	0	–	–
550^t	do	Tap	10	Bouillon	0	–	–
550^t	do	do	10	Bile	0	–	–
568	Dec. 8, 1909	Storage	10	Bouillon	0	–	–
568	do	do	10	Bile	0	–	–
568^t	do	Tap	10	Bouillon	0	–	–
568^t	do	do	10	Bile	0	–	–
574	Dec. 11, 1909	Storage	10	Bouillon	10	–	–
574^t	do	Tap	10	Bile	38	–	–
576	Dec. 13, 1909	Storage	10	Bouillon	0	–	–
576	do	do	10	Bile	0	–	–
576^t	do	Tap	10	Bouillon	0	–	–
576^t	do	do	10	do	0	–	–
578	Dec. 14, 1909	Storage	10	Bile	46	+	–
578^t	do	Tap	10	Bouillon	18	+	–
580^t	Dec. 15, 1909	do	10	Bile	14	–	–
584	Dec. 17, 1909	Storage	10	Bouillon	([a])	–	–
588	Dec. 20, 1909	do	10	do	0	–	–
588	do	do	10	Bile	0	–	–
588^t	do	Tap	10	Bouillon	0	–	–
588^t	do	do	10	Bile	0	–	–
594	Dec. 23, 1909	Storage	10	Bouillon	31	+	–
594	do	do	10	Bile	52	+	–
594^t	do	Tap	10	Bouillon	30	+	–
594^t	do	do	10	Bile	57	+	–
596	Dec. 27, 1909	Storage	10	do	50	+	–
596^t	do	Tap	10	do	38	+	–
600^t	Dec. 29, 1909	do	10	Bouillon	33	+	–
600^t	do	do	10	Bile	60	+	–
602	Dec. 30, 1909	Storage	10	Bouillon	31	+	
602^t	do	Tap	10	do	29	+	–
604	Dec. 31, 1909	Storage	10	Bile	65	+	–
604^t	do	Tap	10	Bouillon	37	+	– [b]

[a] Bubble.

[b] Bacillus isolated which was agglutinated by typhoid agglutinating serum, but proved not identical with Pseudomonas protea.

From this table it may be seen that between August 7 and December 31, 1909, a total of 107 fermentation tubes, representing 67 separate samples of filtered water from the storage basin and from a city tap, were examined by a special method adapted to the isolation of any organisms resembling *B. typhosus* in growth on Endo's medium and in agglutinating with antityphoid serum. From nine of these samples an organism was isolated which proved identical with the first agglutinating *Ps. protea* (No. 362^t). From sample No. 424 an organism was isolated which was partially verified as identical with these and is so considered in the table, although the cultures were accidentally destroyed before complete identification. Organisms which were agglutinated very imperfectly by the antityphoid serum used were isolated from samples Nos. 434^t, 468, and 604^t, but these failed to liquefy gelatin, and in other respects resembled the colon bacillus rather than *Ps. protea*. The figures of the above table may be briefly summarized as follows:

Total number of fermentation tubes containing 10 c. c. of the storage reservoir water examined	52
Number of separate samples of storage reservoir water examined	32
Number of samples of storage reservoir water showing presence of *Pseudomonas protea*	5
Total number of fermentation tubes containing 10 c. c. of tap water examined	55
Number of separate samples of tap water examined	35
Number of samples of tap water showing presence of *Ps. protea*	5
Total number of fermentation tubes (lactose bouillon and lactose bile) containing 10 c. c. of filtered water (storage and tap) examined	107
Number of lactose bile fermentation tubes examined	51
Number of lactose bile fermentation tubes in which *Ps. protea* was demonstrated	7
Number of lactose bouillon fermentation tubes examined	56
Number of lactose bouillon fermentation tubes in which *Ps. protea* was demonstrated	3

This summary shows that there has been no difference demonstrated between the samples from the filtered-water reservoir and the samples from a city tap as regards the prevalence of this organism. This is to be expected, as the effluent from the filters is discharged from the filtered-water reservoir after probably not more than twenty-four hours storage there, and as no noteworthy difference exists between the effluent from the filter beds and the water from the city tap in respect to the total bacterial count and the prevalence of *Bacillus coli.* The parity of the waters in the prevalence of *Ps. protea* eliminates, however, the probability that it is especially an inhabitant of the water mains. The results indicate that lactose bile is a more favorable medium than lactose bouillon for the isolation of this organism.

While the figures presented are too small and the intervals between examinations too irregular to warrant definite conclusions as to seasonal variations in the prevalence of *Ps. protea*, they indicate a definite and progressive decrease in prevalence from August to November. Examinations during November and December, although made with greater frequency and regularity than during August, September, and October, were all negative. The following table shows the results of the examinations during these months; comparison with Table No. 1 will show the date of each examination. The figures given as to the total bacterial count (gelatin plates at 20° C., forty-eight hours) and the prevalence of *B. coli* are compiled *not* from the daily examinations made during these months, but including only the samples which were also examined for the presence of *Ps. protea.*

Table No. 2.—*Monthly prevalence of Pseudomonas protea in filtered (storage and tap) water of Washington, D. C., as determined from August 7 to December 31, 1909, compared with the prevalence of B. coli and the average number of bacteria per cubic centimeter as computed from the same specimens.*

	August.	September-	October.	November.	December.
Number of samples of filtered (storage and tap) water examined during the month for Ps. protea	11	6	10	19	21
Number of samples showing the presence of Ps. protea	8	1	1	0	0
Number of samples showing the presence of B. coli	1	0	0	0	11
Number of fermentation tubes examined for Ps. protea	21	8	15	33	30
Number of fermentation tubes showing the presence of Ps. protea	8	1	1	0	0
Number of fermentation tubes showing the presence of B. coli	1	0	0	0	14
Percentage of samples showing the presence of Ps. protea	+72	+16	10	0	0
Percentage of samples showing the presence of B. coli	9	0	0	0	+ 52
Percentage of fermentation tubes showing the presence of Ps. protea	+38	12.5	6.6	0	0
Percentage of fermentation tubes showing the presence of B. coli	4.7	0	0	0	+ 42
Average number of bacteria per cubic centimeter in samples examined	38	40	25	11	+200

It seems worthy of note that the prevalence of this organism in the filtered water does not correspond either to the total number of bacteria per cubic centimeter or the prevalence of *B. coli.* It is also noted that in no case has this organism been isolated from a fermentation tube from which *B. coli* or any other aerobic lactose-fermenting organism was isolated. This is not altogether remarkable as regards the samples of filtered water, of which so few showed the presence of *B. coli;* but it perhaps has some significance in explaining the failure to isolate the same organism from the unfiltered water and from stools.

EXAMINATION OF UNFILTERED POTOMAC RIVER WATER FOR THE PRESENCE OF ORGANISMS AGGLUTINATED BY ANTITYPHOID SERUM.

The water which is supplied to Washington is received from the Potomac River into Dalecarlia reservoir, passed thence into the Georgetown reservoir, and thence into the larger Washington reservoir, where it undergoes short storage and sedimentation before being applied to the filters. Dalecarlia reservoir is, however, frequently by-passed, in which case the river water is received directly into the Georgetown reservoir. The specimens designated "raw water" were collected from the inlet of Dalecarlia reservoir, or, when this was by-passed, from the inlet of Georgetown reservoir. Specimens of "applied water" were collected at the filtration plant from the Washington reservoir, as being applied to the filters.

Of the raw water, 126 samples were examined from June 7 to December 31, 1909, by the technic already described for the isolation of organisms growing like *B. typhosus* on Endo's medium and agglutinated by typhoid agglutinating serum. During the same period 108 samples of applied water were similarly examined. The majority of the samples of both raw and applied water examined were 10 c. c. samples in lactose bile fermentation tubes, a small proportion being 0.1 c. c., 1 c. c., and 10 c. c. samples in ordinary lactose bouillon fermentation tubes.

The colon bacillus was demonstrated in 89 per cent of the samples of the raw water examined and in 50 per cent of the samples of applied water. The average bacterial count in these specimens was as follows:

	Bacteria in 1 c. c.						
	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Raw water	2,400	1,325	926	1,088	310	320	24,500
Applied water	610	490	460	280	180	142	475

The result of all the examinations of raw and applied water for *Ps. protea* was negative. A number of organisms which were agglutinated more or less completely by the typhoid agglutinating serum used were isolated from both the raw and applied water, but these organisms could in every case be differentiated from *Ps. protea*. They all failed to liquefy gelatin, and produced acid and gas in both lactose and dextrose bouillon; and while none of them have been completely identified, yet they may all be considered as belonging to the colon group. So far as studied they have all shown more feeble agglutinating reactions with typhoid immune serum than does *Ps. protea*. By the technic employed agglutinating organisms were selected from clear, colorless, cr pinkish colonies on Endo's medium. As strains of *B. coli* or organisms very closely allied culturally not infrequently form such colonies on Endo's medium, and as strains of colon bacilli and allied species are also not infrequently agglutinated to a slight extent by typhoid agglutinating serum, it is not remarkable that several such agglutinating strains were encountered in the course of these examinations.

As regards the failure to isolate from the unfiltered water the same agglutinating organism as was isolated quite commonly by the same technic from the filtered water, the following hypotheses may be advanced in explanation:

1. That this organism is absent from the water before filtration. In this case it is necessary to assume that the organism is descended from some organism present in the unfiltered water and undergoes biological changes in the filter beds or in the filtered water, acquiring there the properties which distinguish it from its ancestor; or else, which seems less probable, that it is introduced into the filtered water

from some unknown source other than the unfiltered water and undergoes multiplication there.

2. That the same organism is present but relatively rare in the unfiltered water. In this case the relatively greater abundance in the filtered water may be explained by the easier passage of this particular organism through the filter beds or by rapid multiplication either in the filter beds or in the filtered water.

3. That the organism is present in the unfiltered water in equal or perhaps greater numbers than in the filtered water. Failure to isolate it must in this case be attributed to the technic employed being unsuitable. It seems not improbable that the conditions encountered in a fermentation tube of lactose bouillon or lactose bile, incubated at 40° C., may be comparatively unfavorable for the development of this organism, and that other organisms which are more common in the unfiltered than in the filtered water may constantly overgrow it. As the unfiltered water contains from ten to one hundred times as many bacteria per cubic centimeter as the filtered water, the chances of overgrowth must be proportionately greater in this water. The colon bacillus, for the growth of which the conditions in the fermentation tube are especially adapted, suggests itself as one of the organisms most likely to overgrow this organism.

It should be possible by experimental work to determine the conditions especially favoring the growth of *Ps. protea*, and by employing a technic based on these considerations to arrive at more definite conclusions concerning the general prevalence of the organism. However, it has been impossible up to this time to undertake any such research.

PREVALENCE IN WELL AND SPRING WATERS.

Samples of water from twelve wells and springs in and around the District of Columbia have been examined at various times by the same technic. The samples represented waters varying in bacteriologic quality from pure to very highly polluted. From two of the wells organisms were isolated which agglutinated in quite high dilutions (1:1000 and 1:2000) with typhoid agglutinating serum; but both of these organisms produced gas from lactose, failed to liquefy gelatin, and were otherwise differentiated from *Ps. protea.*

GENERAL PREVALENCE IN NATURE.

The only evidence which can be offered on this point is the negative evidence derived from the examination of a large number of stools and urine of typhoid fever patients, convalescent and recovered typhoid fever patients, and normal persons, by plating out on Endo's medium. In the Hygienic Laboratory more than 2,000 specimens of stools and urine have been examined during 1907, 1908, and 1909.

As the appearance of *Ps. protea* colonies on Endo's medium is very similar to the appearance of typhoid colonies, such colonies would be likely to be fished and tested; and as all organisms agglutinated by typhoid immune serum are identified culturally, it seems almost inevitable that this organism should have been recognized had it appeared on the Endo plates with any considerable frequency. The fact, too, that this organism has not been reported by other observers in their examination of stools for typhoid bacilli is evidence against its being demonstrable in stools by the methods usually employed for the isolation of the typhoid bacillus.

SUMMARY.

As regards the prevalence of this organism, then, it may be said that it has been found to be more common than *B. coli* during the months of August, September, and October, 1909, in the filtered Potomac River water supplied to Washington—water of good quality as judged by standard bacteriologic and chemical methods. A large number of examinations of the unfiltered water, which shows a much higher bacterial count and an almost constantly demonstrable sewage pollution, has failed to show the presence of this organism. Technic similar to that employed in the examination of water has failed to demonstrate this organism in any of a large number of stools examined. All of this evidence, so far as it goes, is against this organism being of fecal origin, pointing rather to a saprophytic nature. Since, however, the method employed in all of these examinations is a selective method, especially adapted for the growth and isolation of organisms of the colon-typhoid group, the failure to isolate *Ps. protea* in the presence of these organisms can not be taken as proving its absence there.

SPECIFICITY OF AGGLUTINATION IN GENERAL.

Since attention was first called by Gruber and Durham in 1896 to the fact that in certain bacterial infections the serum of the infected animal develops in high degree the property of agglutinating the specific infecting organism, an enormous amount of research has been devoted to the many phases of this phenomenon, accumulating a voluminous literature, extremely confusing by reason of the apparently conflicting results of different observers. Much fairly definite knowledge of this phenomenon has, however, resulted; certain broad, underlying principles have been demonstrated, and theories formulated which are reasonably satisfactory in explaining and harmonizing the observations recorded.

Of the many questions regarding the phenomenon, the one of perhaps the greatest practical importance both to the clinician and to the bacteriologist is the question of specificity. Upon the determination of this point depends the diagnostic value for the clinician of the agglutinating reactions of his patients' serum; and the determinative value for the bacteriologist of the agglutination of bacteria by serum derived from animals inoculated with known varieties of bacteria. The numerous researches made upon this point, so far from settling the question absolutely, have demonstrated that it is one which must be determined in each case by the consideration of various factors. A classic review of the subject of agglutination, with bibliography to date, is given by Paltauf (9) in Kolle and Wassermann's "Handbuch der pathogenen Mikroorganismen."

Following is a brief consideration of the factors involved in determining the specificity of an agglutinating serum, being in the main a condensation from Paltauf's extensive work:

It may be assumed that in any agglutinating "immune" serum the principal agglutinin (*Hauptagglutinin*) possesses receptors corresponding to the specific receptors of the bacterial cell causing the production of this agglutinin. In addition, there are more or less "partial agglutinins," corresponding to bacterial cell receptors which are common to the specific bacterium and to some other bacteria. The term "group agglutinin" is commonly used to designate such a partial agglutinin. It does not, however, necessarily signify that the bacteria concerned belong to the same biologic group. Different immune sera show variations in their relative amounts of principal agglutinin and partial agglutinins, depending on the species or individual strain of bacteria giving rise to the formation of the agglutinin, the species and individuality of the animal organism producing it, the duration of infection, etc.

Adapting the schematic illustration given by Durham (10) in his exposition of the theory, this may be represented as follows:

(1) Receptors of *B. typhosus* = A, B, C, D, E;
(2) Receptors of *B. coli* = D, E, F, G, H;
(3) Receptors of specific agglutinin for *B. typhosus* = a, b, c, d, e;

Reaction between (1) and (3) = Aa, Bb, Cc, Dd, Ee; representing combination between the "principal agglutinin" and the specific agglutinable substance of *B. typhosus*, involving all receptors of both.

Reaction between (2) and (3) = a, b, c, Dd, Ee, F, G, H; representing combination between the partial agglutinin (d, e) for *B. coli* contained in the serum, and such bacterial receptors (D, E) as are common to *B. typhosus* and *B. coli.*, leaving uncombined some of the receptors (a, b, c) of the principal agglutinin, and some of the receptors (D, E, F) of the colon bacillus.

The bacteria for which the principal agglutinin is specific would always be agglutinated in higher dilution than the bacteria combining with only a portion of the agglutinin (partial agglutinin) provided that the agglutinability of the respective bacteria were always equal. This, however, is not always the case. As different strains of the same bacteria may vary widely in the limit of their agglutinability by a given specific serum, so may two species of bacteria differ in this respect. It would seem that as between two species of bacteria the greater agglutinability of one does not necessarily imply combination with a greater number of receptors. In other words, that the degree of agglutination produced is not necessarily an index of the amount of chemical combination taking place between cell and agglutinin receptors. As illustrating this may be taken the not infrequent cases of typhoid fever in which the serum agglutinates some other organism, as *B. paratyphosus*-B, in higher dilution than the specific infecting organism, *B. typhosus*.

In such cases the principal agglutinin may often be differentiated from the partial agglutinin by saturation of the serum with the respective agglutinating organisms, as demonstrated by Castellani (11). According to the law deduced by Castellani, saturation with the organism specific for the principal agglutinin removes from the serum all the agglutinins for both organisms, while saturation with the organism which is agglutinated only by the partial agglutinin removes only those receptors which constitute its own partial agglutinin, diminishing, perhaps, but not completely removing the agglutinating power of the principal agglutinin for its specific bacterial organism.

The laws deduced by Castellani have not proved invariably true. Park (12) after summarizing the results of absorption experiments, concludes that "the absorption method simply proves (therefore) that when one variety of bacteria removes all agglutinins for a second, the agglutinins in question were not produced by that second variety." To this extent the absorption of agglutinins gives valuable information in regard to the specificity of an agglutinative serum.

Besides the partial agglutinins which may be present in any immune serum, there may also be present other *heterologous* agglutinins, possessing no receptors in common with the specific or principal agglutinin, and having therefore no power of combining with the organism which caused the production of this specific agglutinin. It has been shown by Bordet,(13) by Posselt and Sagasser,(14) and by Hetsch and Lentz(15) that normal serum may possess numerous such heterologous agglutinins, affecting independently an indeterminate number of widely different bacteria; and that the increase of the agglutinin specific for a given bacterial organism in the course of infection or immunization may be accompanied by an increase of the heterologous agglutinin which agglutinates some other totally different bacterium. In this case, saturation with the organism specific for the principal agglutinin does not remove the heterologous agglutinin, showing that the latter is not necessarily bound to the principal agglutinin.

Wilson and Symmers (16) and Wilson (17) have recently reported some striking instances of heterologous agglutination. Wilson found that the blood of 90 per cent of 184 cases of epidemic cerebro-spinal meningitis agglutinated in quite high dilution a bacillus (*B. aquatilis alcaligenes*) isolated from Belfast tap water, closely related to *B. fecalis alcaligenes*, and having in all probability no etiologic connection with the disease. Saturation of this serum with meningococci removed the agglutinin for the meningococcus, but failed to remove the agglutinin for *B. aquatilis alcaligenes*. *B. typhosus* was also agglutinated by the serum of a number of these cases. Further, the serum of cases of typhus fever was found to agglutinate quite remarkably both *B. typhosus* and a bacillus of the colon group isolated from the stools of one of the cases of typhus fever, but having no demonstrable etiologic relation to the disease. A quite extensive review of the literature of heterologous agglutination may be found in Wilson's article.

Two essentially heterologous agglutins may, theoretically at least, possess some common receptors, which possibility may affect to some extent the results obtained by saturation.

In the case, then, where a serum is found to agglutinate more than one variety of bacteria, there are several possibilities:

1. The presence of more than one specific agglutinin, explained by mixed infection.

2. The presence of a single specific agglutinin, with partial agglutinins for other bacteria.

3. The presence of separate agglutinins which are heterologous toward each other.

4. A combination of two or more of the above.

The determination of the actual case is, therefore, a matter of considerable difficulty, even supposing that the theories above given correspond fully to the facts, whereas it is only assumed that the theories are at best roughly diagrammatic.

Paltauf, in summarizing, concludes that the specificity of an agglutinating serum depends upon—

1. The kind of bacterium; whether this possess a specific receptor apparatus. If the agglutinable substance is variable and a specific receptor apparatus wanting, then agglutination is to be regarded not as a specific characteristic but rather as an individual peculiarity of the "strain," or of those strains with the same antecedents.

2. The animal organism furnishing the antibody, taking into consideration not only the species but likewise the individuality of the animal.

3. The strength of the agglutination; the strength of the principal agglutinin being taken as an indicator of the specificity. The agglutinating strength of the principal agglutinin requisite for the serum to be considered determinative can not be stated absolutely; it should agglutinate the specific organism in sufficiently high dilution to eliminate the probability of heterologous or group agglutination being responsible for the reaction. The fact that a serum of high specific potency exhibits in low dilution a group or heterologous agglutination with other bacteria, does not invalidate the specificity of the serum.

It is quite possible, as explained above, that an easily agglutinable variety of bacteria may be agglutinated by the partial agglutinin of an immune serum in actually higher dilution than a poorly agglutinable strain of the specific organism is agglutinated by the principal agglutinin. This factor must, of course, be determined experimentally in each case.

The source of error in drawing conclusions as to specific infections from the agglutinations produced by human serum, such as must be used for clinical diagnosis, must necessarily be greater than with the more highly potent and more specific serum obtainable by inoculation of animals. Valuable information can be obtained, however, in the diagnosis of many diseases, by a close study of the agglutinating reactions; information which under favorable circumstances is, if properly considered, almost certain in diagnostic value.

INTERAGGLUTINATION BETWEEN B. TYPHOSUS AND BACILLI OF THE PROTEUS GROUP.

A number of observations have been made on the agglutinating properties of various strains of bacilli of the proteus group. These observations have shown an occasional and inconstant relationship in agglutinating properties between bacilli of the proteus group and *B. typhosus*. In view of the close biologic relationship between *Ps. protea* and the members of the proteus group, a brief review of this literature is considered germane to this paper, more especially since the descriptions given of many of the "proteus bacilli" are insufficient to differentiate them definitely from *Ps. protea*. For instance, *Proteus fluorescens*, described by Jaeger as the specific cause of acute febrile icterus (Weil's disease), has since been classified by Migula as a pseudomonas, although originally described by Jaeger as being a bacillus having peritrichic flagella.

It was reported at least as early as 1896, by Lannelongue and Achard (18), that the serum of animals injected with cultures of

Proteus vulgaris developed increased agglutinating power for the injected organism. They recognized that the agglutination was often more marked with the strain of proteus injected than with other strains of proteus, and was sometimes limited altogether to the homologous strain. Grassberger, (19) in 1896, reported a strong agglutination of *Proteus vulgaris* by the serum of a patient suffering from a suppurative proteus infection.

These observations have since been amply confirmed, both clinically and experimentally.

AGGLUTINATION OF B. TYPHOSUS BY PROTEUS SERUM.

Lubowski and Steinberg, (20) in 1904, reported a case of mixed proteus and staphylococcus infection of the mastoid cells, in which *B. typhosus* was agglutinated by the patient's serum in a dilution of 1:40: also another case of pure proteus infection of the mastoid, with abscess of the brain, in which *B. typhosus* was agglutinated in dilution of 1:80. In both of these cases the infecting proteus was also agglutinated by the patient's serum, in higher dilutions than the typhoid bacillus. By injecting animals (rabbits and guinea pigs) with pure cultures of proteus bacilli of various origins, these authors were able to procure sera which agglutinated their proteus strains in dilutions of 1:5,000 to 1:80,000, and agglutinated *B. typhosus* in dilutions of 1:80 to 1:1,280. In no case was the agglutinating strength of any proteus-immune serum for *B. typhosus* more than one-fourth of its strength for *Proteus vulgaris*, and in most cases it was much less than this.

Jochmann (21) confirms the above by his report of a similar case of suppurative mastoid infection, resulting in a sinus thrombosis and general sepsis. *Proteus vulgaris* and *streptococci* were isolated from the blood during life. The serum of this patient agglutinated *B. typhosus* 1:160, and *Proteus vulgaris* (homologous strain), 1:640. From rabbits immunized against this strain of proteus, a serum was obtained which agglutinated the homologous proteus strain 1:5,000 to 1:10,000, and *B. typhosus* 1:80 to 1:640.

Zupnik, (22) Eckhardt, (23) and others have reported marked agglutination of the typhoid bacillus by the serum of persons suffering from Weil's disease (acute febrile icterus). This reaction has been ascribed by some to the presence of bile in the serum. Jaeger (24) has described a bacillus, which he considers a variety of proteus, as the specific cause of this disease, and although this etiology is not universally accepted, it offers a plausible explanation of the agglutination of *B. typhosus* in this disease, as a group agglutination—an explanation already offered by a number of writers. Lüdke, (25) after a review of the literature and quite extensive original researches on the agglutination of *B. typhosus* in cases of icterus of various origin and degree, concludes that the agglutination bears no relation to the degree of icterus, but is relatively more common in catarrhal icterus, which may be supposed to be due to bacterial infection. In his two cases of Weil's disease, Lüdke found both *B. typhosus* and *Proteus vulgaris* agglutinated by the serum of the patients at 1 : 20 or higher.

The agglutination of *B. typhosus* in proteus infections is not constant, however. Pfaundler (26) obtained negative results as regards agglutination of *B. typhosus* in a case of demonstrated proteus infection: and the agglutination of *B. typhosus* in Weil's disease has likewise been found not constant.

AGGLUTINATION OF PROTEUS VULGARIS BY THE SERUM OF PERSONS AND ANIMALS INFECTED WITH B. TYPHOSUS.

Lannelongue and Achard, (18) in their previously quoted article, report that they found proteus vulgaris agglutinated in some cases by the serum of persons suffering with ulcerative diseases of the intestines, such as typhoid fever and carcinoma of the rectum. They give no details as to the number of cases observed, the proportion of cases giving a positive reaction, the dilutions in which the serum agglutinated *B. proteus*, the cultural characteristics of the proteus strains, or the technic employed. Rodella, (27) in an experimental investigation of the agglutination reactions of *Proteus vulgaris*, found it agglutinated by three out of six cases of typhoid fever. The dilution in which the agglutination occurred is not given; but it is stated that the agglutination was not nearly so characteristic with proteus as with *B. typhosus*.

Haim (28) has reported a case, clinically typhoid fever, in which the stools in the early stages of the disease, exhibited *Proteus vulgaris* to the exclusion of the usual intestinal flora. The proteus bacillus isolated from the stools was agglutinated by the serum of this patient in dilution of 1 : 50; *B. typhosus* was agglutinated in dilution of 1 : 100. The author regards this as a case of mixed infection, a conclusion which seems justified by the facts.

Beco (29), working with a typhoid-immune serum which agglutinated *B. typhosus* in dilutions of from 1 : 10,000 to 1 : 100,000, found that it also agglutinated various strains of proteus bacilli in dilutions of from 1 : 100 to 1 : 1,000.

On the other hand, uniformly negative results as regards the agglutination of *Proteus vulgaris* by the serum of persons having typhoid and paratyphoid fevers, and of animals immunized against *B. typhosus*, have been obtained in tests aggregating a considerable number by Grassberger, (19) Pfaundler, (16) Lubowski and Steinberg, (20) and Jochmann. (21)

AGGLUTINATION OF PROTEUS VULGARIS BY NORMAL SERUM.

The serum of a number of normal persons was tested by Lannelongue and Achard (18) for agglutination with *Proteus vulgaris*, the results being negative with but one exception. The number of tests made and the dilutions employed are not stated. Pfaundler (30), who made an extensive study of the agglutinating reactions of the serum of young children toward organisms isolated from their own and others' stools, found *Proteus vulgaris* agglutinated in only two cases—one a typical case of Weil's disease, and one an unexplained case of intestinal catarrh. He attributes the agglutination of proteus in both these cases to probable infection with proteus bacilli; but he states that the serum of older persons frequently agglutinates proteus bacilli in dilutions of 1 : 10 to 1 : 20.

Rissling (31) found *B. typhosus* and *Proteus vulgaris* agglutinated by the serum from normal animals as follows:

	Proteus vulgaris.	B. typhosus.
Horse serum (normal)	1 : 10	1 : 60
Ox serum (normal)	1 : 100	1 : 30
Sheep serum (normal)	1 : 20	1 : 20
Hog serum (normal)	1 : 10	1 : 10

Müller (32) (quoted by Rissling) found *Proteus vulgaris* agglutinated by normal animal serum as follows:

Normal serum from—	Samples examined.	Highest dilution of serum in which agglutination was positive.				
		1 : 8.	1 : 16.	1 : 32.	1 : 64.	1 : 128.
Horse	5	....	....	1	1	3
Ox	5	1	1	1	....	2
Sheep	5	....	....	3	1	1
Hog	4	....	....	....	1	3

The review of the above literature shows:

1. That bacilli of the *proteus vulgaris* group can produce, in both clinically and experimentally infected animals, agglutinins whose action, however, is apt to be limited to individual strains of proteus. The failure to produce agglutinins having a constant and characteristic action upon the whole species may be due to (*a*) variability in the agglutinable substance within an otherwise identical species, or (*b*) nonidentity of the so-called *Proteus vulgaris* bacilli used by various observers, or by the same observer in different experiments. Certainly the descriptions given by most of the above-quoted authors are insufficient for accurate identification.

2. That the serum of persons and animals infected with varieties of proteus bacilli sometimes causes definite agglutination of *B. typhosus*. Whether this is to be regarded as a partial (group) agglutination or a heterologous agglutination remains to be demonstrated.

3. That the serum of persons having typhoid fever has, in comparatively few instances, been found to agglutinate bacilli of the proteus group; less strongly and less characteristically, however, than *B. typhosus*. It has not been demonstrated whether the agglutination of proteus bacilli in such cases is caused by specific proteus agglutinins due to mixed intestinal infection or by the action of partial (group) agglutinins constituting a part of the specific typhoid agglutinins.

4. That the serum of normal persons and of normal lower animals sometimes agglutinates proteus bacilli, usually in low dilutions, just as the same serum agglutinates many other varieties of bacteria.

THE AGGLUTINATION REACTIONS OF PSEUDOMONAS PROTEA.

The agglutinating properties of *Ps. protea* have been studied chiefly in regard to their relation to the agglutinins of *B. typhosus*. These studies have comprised experiments with—

A. Human Serum.

1. Serum of persons having typhoid or paratyphoid fever.
2. Serum of persons having diseases other than typhoid fever.
3. Serum of normal persons.
4. Serum of persons vaccinated with *B. typhosus*.

B. Animal Serum.

1. Serum of normal animals.
2. Serum of animals immunized against *B. typhosus*.
3. Serum of animals immunized against *Ps. protea*.

TECHNIC EMPLOYED.

The first agglutination tests were made with 24-hour bouillon cultures of *B. typhosus* and *Ps. protea*, but it was soon found desirable to work with live 24-hour agar cultures suspended in normal salt solution, consequently the great majority of the tests made were performed with such suspensions. The agar slant cultures of *Ps. protea* used for making these suspensions were planted usually from a stock culture on agar, which was kept at 15° C. and transplanted not oftener than once a month. The agar cultures of *B. typhosus* were planted either from a similar stock culture or from young agar or bouillon cultures. Cultures in both cases were incubated at 37° C. for eighteen to twenty-four hours.

Agglutination tests were made in small test tubes, at room temperature except when otherwise noted, and were observed for macroscopic agglutination. In a considerable number of cases miscroscopic examinations of the specimens were also made to check the macroscopic observations, and the observations in such cases were always found to agree.

In making dilutions measurements were made, except in special cases, by drops from Pasteur pipettes of as nearly as possible equal caliber. While extreme accuracy can not be obtained in this way, the method is sufficiently accurate for all practical purposes. The volume of fluid used in each tube was always sufficient to make macroscopic agglutination clearly evident. In tests made with the serum obtained from human cases of typhoid fever, agglutination was not recorded as positive unless macroscopically visible within one hour in a dilution of 1 : 40 or higher, these limits being adopted because they are the usual limits set down for determining clinically the Widal reaction. In other experiments, however, it was considered advisable to continue observations longer than one hour. When observations were so continued the fact is noted in the tables following. Controls were made of both *B. typhosus* and *Ps. protea* in all cases, using the same cultures as in the agglutination tests, but without the addition of serum.

The same culture of *B. typhosus* was used in all of the experiments, a stock culture designated "Hopkins," which has been used in the Hygienic Laboratory for several years, is quite constant in all of its characteristics, and is readily agglutinable. In the first experiments various culturally identical strains of *Ps. protea* (Nos. 362^t, 366, 366^t, and 368) were used, and while no constant nor considerable difference could be made out between them, two strains, Nos. 368 and 370, were finally selected as being the most representative and most constant.

AGGLUTINATION OF B. TYPHOSUS AND PS. PROTEA BY THE SERUM OF TYPHOID FEVER CASES.

Agglutination tests have been made with the serum from 52 cases of suspected typhoid fever. These specimens of serum were collected in the course of an independent investigation by Passed Asst. Surg. T. B. McClintic, to whom I am also indebted for the results of blood-culture and other clinical data concerning the cases. The clinical course of each case was very carefully followed by Doctor McClintic, so that the diagnoses recorded as typhoid fever, even when not confirmed by bacteriologic findings, may be considered as established beyond a reasonable doubt. It was, unfortunately, not possible in all cases to determine the limit of agglutination for *B. typhosus* and *Ps. protea*, in some cases because the amount of serum available was insufficient, and in other cases because of insufficient time. The following table shows these experiments in detail:

TABLE No. 3.—*Results of agglutination tests with the serum of cases of suspected typhoid fever.*

[+ = Agglutination. ± = Doubtful agglutination. 0 = No agglutination. — = No test. bl = Bouillon.]

No.	Date of collection of blood specimen.	Date of examination of specimen.	Day of disease when specimen was collected.	Clinical diagnosis.	Result of blood culture.	Agglutination with bacillus typhosus "Hopkins."					Agglutination with protea Ps. protea.					Culture of Ps. protea used.	Remarks.
						Serum dilutions.											
						1/40.	1/80	1/160.	1/320.	1/640.	1/40.	1/80.	1/160.	1/320.	1/640.		
1	Aug. 23	Aug. 24	Third week	Typhoid	Typhoid	[a] +	—	—	—	—	+	—	—	—	—	366 bl.	
2	do.	do.	Fourth week	do.	Negative	[a] +	—	—	—	—	+	—	—	—	—	366 bl.	
3	do.	do.	Second week	Uncertain	(b)	0	—	—	—	—	0	—	—	—	—	366 bl.	
4	Aug. 26	Aug. 26	Sixteenth day	Typhoid	Typhoid	+	+	+	+	+	0	0	0	0	0	366^{t} bl.	
5	Aug. 24	do.	Third week	do.	Negative	+	+	±	—	—	+	+	+	—	—	366 bl.	
6		do.	(?)	Not typhoid	None	0	—	—	—	—	0	—	—	—	—	366 bl.	
7	Aug. 24	do.	Ninth day	Typhoid	Typhoid	+	—	—	—	—	+	—	—	—	—	366 bl.	
8	Aug. 27	Aug. 30	Thirteenth day.	do.	do.	+	—	—	—	—	+	—	—	—	—	368 bl.	Also 366B bl. positive Widal Aug. 25 by another observer.
9	Aug. 21	do.	Ninth day	do.	Negative	0	—	—	—	—	0	—	—	—	—	368 and 366B bl.	
10	Aug. 27	do.	Fifth day +	do.	do.	+	—	—	—	—	+	—	—	—	—	368 and 366B bl.	
11	Aug. 31		First week	do.	Typhoid	+	±	0	—	—	+	0	0	—	—	368 bl.	
12	do.		Twelfth day	do.	do.	0	0	0	—	—	0	0	0	—	—	368 bl.	
13	do.	Sept. 1	First week	do.	Negative	0	—	—	—	—	0	—	—	—	—	362^{t} bl.	Positive Widal Sept. 20.
14	do.	do.	Fifteenth day +.	do.	Typhoid	+	+	+	—	—	+	±	0	—	—	362^{t} bl.	
15	do.	do.	do.	do.	do.	0	—	—	—	—	0	—	—	—	—	362^{t} bl.	
16	do.	do.	Eleventh day	do.	Negative	±	—	—	—	—	±	—	—	—	—	362^{t} bl.	
17	Sept. 1	Sept. 3	(?)	Endometritis	do.	0	—	—	—	—	0	—	—	—	—	368	
18	do.	do.	Eighth day	Typhoid	do.	+	+	+	0	—	+	+	+	+	—	Agar	
19	Sept. 3	Sept. 4	Fifth day	do.	Typhoid	0	—	—	—	—	+	—	—	—	—	Agar	
20	Sept. 2	do.	Fifth day +	Not typhoid	None	0	—	—	—	—	0	—	—	—	—	Agar	Slight gastro-intestinal derangement.
21	Sept. 3	do.	Sixth day +	Malaria	do.	0	—	—	—	—	0	—	—	—	—	Agar	Widal negative Oct. 30.
22	do.	do.	Fourteenth day.	Typhoid	Negative	+	+	+	+	0	+	+	+	±	0	Agar	
23	Sept. 8	Sept. 8	Sixth day	Malaria	None	0	—	—	—	—	0	—	—	—	—	Agar	
24	do.	do.	(?)	do.	do.	0	—	—	—	—	0	—	—	—	—	Agar	
25	do.	do.	Twelfth day	Typhoid	Typhoid	—	+	—	—	+	+	0	—	—	—	Agar	

[a] Test made by another observer using a bouillon culture of B. typhosus.

[b] An organism culturally resembling B. proteus vulgaris isolated from blood; possibly contamination.

TABLE No. 3.—*Results of agglutination tests with the serum of cases of suspected typhoid fever*—Continued.

No.	Date of collection of blood specimen.	Date of examination of specimen.	Day of disease when specimen was collected.	Clinical diagnosis.	Result of blood culture.	Agglutination with bacillus typhosus "Hopkins."					Agglutination with protea Ps. protea.					Culture of Ps. protea used.	Remarks.
						Serum dilutions.											
						1:40.	1:80.	1:160.	1:320.	1:640.	1:40.	1:80.	1:160.	1:320.	1:640.		
26	Sept. 8	Sept. 9	Sixth day	Typhoid	Typhoid	0	–	–	–	–	0	–	–	–	–	Agar	
27	...do	...do	Twentieth day +.	Typhoid or paratyphoid.	None	0	–	–	–	–	+	+	–	0	–	Agar	
28	...do	...do	Tenth day	Typhoid	Typhoid	0	–	–	–	–	±	–	–	–	–	Agar	Positive Widal 1 : 40, obtained Sept. 8, by another observer.
29	Sept. 9	Sept. 10	Third month	...do	Negative	–	+	+	+	+	–	0	0	0	0	Agar	
30		Sept. 11	(?)	Tuberculosis	None	0	–	–	–	–	0	–	–	–	–	Agar	Tubercle bacilli in sputum.
31		...do	First week	Malaria	Negative		–	–	–	–	0	–	–	–	–	Agar	
32	Sept. 13	...do	Tenth day	Typhoid	Tyhpoid	±	–	–	–	–	+	–	–	–	–	Agar	
33	...do	...do	Fifteenth day	...do	...do	±	0	0	0	0	+	+	±	0	0	Agar	
34	Sept. 15	Sept. 15	Third week	Para-typhoid B	Negative	0	0	–	–	–	±	0	–	–	–	Agar	Serum agglutinated para-typhosus A and B, 1 : 40, Sept. 14.
35	...do	...do	Fourteenth day.	Typhoid	Typhoid	0	0	–	–	–	+	±	–	–	–	Agar	
36	Sept. 18	Sept. 18	Sixth day +	...do	Negative	0	–	–	–	–	0	–	–	–	–	Agar	Oct. 13, positive agglutination with paratyphoid A.[a]
37	Sept. 17	...do	Fifth day	Pneumonia	...do	0	–	–	–	–	0	–	–	–	–	Agar	
38	...do	...do	Fourth day +.	Miliary tuberculosis	...do	[a]+	0	–	–	–	–	0	–	–	–	Agar	Previous attack of typhoid 3 years ago.
39	Sept. 18	...do	Fifteenth day +.	Typhoid	...do	+	+	+	±	0	+	+	+	+	0	Agar	Negative to para B.[a]
40	Sept. 20	Sept. 23	Tenth day	...do	...do	0	–	–	–	–	+	+	–	–	–	Agar	Positive Widal, 1 : 40, Sept. 30.[a]
41	...do	...do	Twelfth day	...do	None	0	0	–	–	–	+	+	+	–	–	Agar	
42	Sept. 23	...do	Twenty-second day.	...do	...do	0	0	0	–	–	+	+	0	–	–	Agar	Positive Widal, 1 : 40, Oct. 11.[a]
43	...do	...do	Sixth week	...do	...do	+	+	+	–	–	+	+	+	–	–	Agar	Negative agglutination with para B, 1 : 40.
	Oct. 2	Oct. 4	Seventh week	...do	...do	+	+	+	+	0	+	+	+	±	0	Agar	
44	Sept. 25	Sept. 27	(?)	Not typhoid	Negative	0	–	–	–	–	0	–	–	–	–	Agar	
45	...do	...do	(?)	Pulmonary tuberculosis.	None	0	–	–	–	–	0	–	–	–	–	Agar	Tubercle bacilli in sputum.

46	...do.....	...do.....	Twenty-first day.	Typhoid..............	...do......	+	0	0	−	−	+	+	+	±	−	Agar....	Negative to para B, 1 : 40, Sept. 27.[a]
47	...do.....	...do.....	Fourteenth day.	.....do................	...do......	0	−	−	−	−	+	−	−	−	−	Agar....	Positive Widal, 1 : 40, Oct. 15.[a]
48	Sept. 26	Sept. 29	First week....	.....do................	Negative..	0	−	−	−	−	+	−	−	−	−	Agar....	B. typhosus in urine.
49	Sept. 29	...do.....	.....do........	.....do................	Typhoid..	0	−	−	−	−	+	+	0	−	−	Agar....	Negative to para B, 1 : 40, Sept. 29.[a]
50	...do.....	...do.....	Tenth day +..	Para-typhoid A.......	Para-typhosus A.	0	−	−	−	−	+	+	0	−	−	Agar....	Negative to para B; positive to para A, 1 : 40, Sept. 29.
51	...do.....	...do.....	Twenty-second day +.	Typhoid..............	Negative..	0	−	−	−	−	+	+	+	+	0	Agar....	B. typhosus in urine, Oct. 15.
52	Oct. 1	Oct. 2	Eighteenth day.	Typhoid or para-typhoid.	....	[a]0	−	−	−	−	+	+	+	+	−	Agar....	Positive to para A and para B, 1 : 100, Oct. 1.

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[a] Test made by another observer using a bouillon culture of B. typhosus.

Summarizing this table—

Total number of cases whose serum was examined	52
Cases of typhoid fever	35
Cases of probable para-typhoid, Type A	2
Cases of probable para-typhoid, Type B	2
Cases of diseases other than typhoid and para-typhoid fevers	13

TYPHOID FEVER CASES.

As is shown by the table, a number of the cases of typhoid fever failing to give the Widal reaction with *B. typhosus* at the first examination, gave a positive reaction at a later date. The results of these later tests are, however, omitted in the following summary, because no corresponding tests were made with *Ps. protea*. Only those specimens are included which were tested at the same time against *B. typhosus* and *Ps. protea:*

	Number.	Per cent.
Cases of typhoid fever examined	35	....
Cases of typhoid fever in which the serum agglutinated B. typhosus, 1 : 40 or higher.	19	54.3
Cases of typoid fever in which the serum agglutinated Ps. protea, 1 : 40 or higher	27	77.0

Classifying the cases of typhoid fever according to the stage of the disease:

	Number.	Per cent.
Cases examined during the first week of illness	8	....
Cases in which the serum agglutinated B. typhosus	2	25.0
Cases in which the serum agglutinated Ps. protea	5	62.5
Cases examined during the second week of illness	14	....
Cases in which the serum agglutinated B. typhosus	7	50.0
Cases in which the serum agglutinated Ps. protea	12	85.7
Cases examined during the third week and fourth week of illness	11	....
Cases in which the serum agglutinated B. typhosus	8	72.7
Cases in which the serum agglutinated Ps. protea	9	81.8
Cases examined later than the fourth week of illness	2	....
Cases in which the serum agglutinated B. typhosus	2	100
Cases in which the serum agglutinated Ps. protea	1	50

From a single examination, then, of the serum from 35 cases of typhoid fever, in various stages, *Ps. protea* was found to be agglutinated in a larger percentage of cases (77 per cent) than *B. typhosus* (54.3 per cent). The greater constancy of the agglutination of *Ps. protea* as compared with the *B. typhosus* is relatively more marked in the first and second weeks of the disease than later.

The following charts show the results of these examinations:

CHART NO. 1.

Showing percentage of cases of typhoid fever in various stages giving positive agglutination reaction (1-40 or higher) with *B. typhosus* and *Ps. protea.*

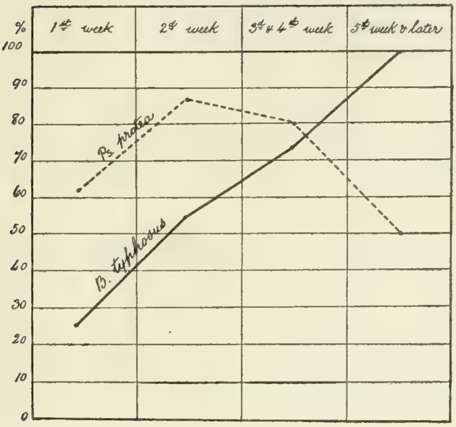
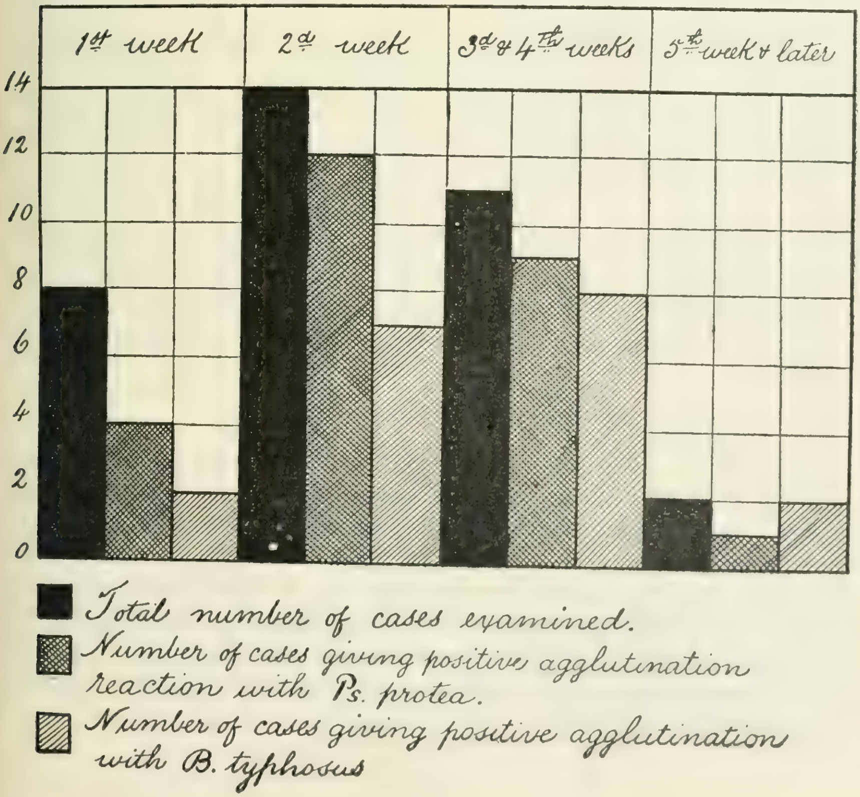


CHART NO. 2.

Showing number of cases of typhoid fever examined in different stages of the disease (by weeks) and number in each stage giving positive agglutination reaction (1-40 or higher) with *B. typhosus* and *Ps. protea.*



As to the relative dilutions in which *B. typhosus* and *Ps. protea* were agglutinated, the cases show wide variation. In some cases (Nos. 4 and 29) *B. typhosus* was agglutinated in dilutions as high as 1 : 640, while *Ps. protea* was not agglutinated even in dilutions of 1 : 40. In other cases (Nos. 41 and 51) *Ps. protea* was agglutinated in dilutions as high as 1 : 160 or 1 : 320, while *B. typhosus* was not agglutinated at all in dilution of 1 : 40. In cases where both organisms were agglutinated, sometimes *B. typhosus* and sometimes *Ps. protea* was agglutinated, in the higher dilution, as shown in the foregoing table.

No opportunity was found to follow the relative development of agglutinins for *B. typhosus* and *Ps. protea* during the course of a case of typhoid fever.

The time limit adopted during which agglutination must take place in order to be regarded as positive, was uniformly one hour. In a few cases which are recorded as negative, agglutination of either *B. typhosus* or *Ps. protea* was noted after a longer interval, but recorded as negative. The relative rapidity of the reaction of agglutination with *B. typhosus* and *Ps. protea* varied greatly. Where both organisms were agglutinated, in general the reaction took place sooner with *B. typhosus* than with *Ps. protea*, but several cases furnished marked exceptions to this.

A quite constant difference was noted between the macroscopic appearance of the agglutinations taking place with *B. typhosus* and those with *Ps. protea*. While *B. typhosus* was almost always agglutinated in large, loose flocculi, *Ps. protea*, even when strongly and quickly agglutinated, formed finer, granular flocculi. Subsequent sedimentation, however, except in the case of weak, partial reactions, was quite complete. The same difference in size and constitution of the flocculi formed by *B. typhosus* and *Ps. protea*, respectively, could be observed under the microscope, where the clumps of *Ps. protea* were seen to be smaller and more compact than the clumps of typhoid bacilli.

CASES OF PARA-TYPHOID FEVER.

Four of the cases examined proved to be probably cases of para-typhoid fever, two being cases of para-typhoid "A" (cases Nos. 27 and 50), and two para-typhoid "B" (cases Nos. 34 and 52), although in only one of these cases was the diagnosis made certain by the isolation of the organism from the blood. In all of these cases the agglutination test was negative with *B. typhosus*, positive with *Ps. protea*, and positive, at some time during the course of the infection, with *B. paratyphosus* A or B; in one case positive with both *B. paratyphosus* A and B.

SERUM OF PERSONS HAVING DISEASES OTHER THAN TYPHOID AND PARA-TYPHOID FEVERS.

The thirteen cases examined as suspected typhoid fever but subsequently diagnosed differently, may be classified as follows:

Malaria	4
Tuberculosis	3
Pneumonia	1
Endometritis	1
Brief febrile affections not further diagnosed	4
Total	13

The serum of all of these cases, tested in a dilution of 1 : 40, failed to agglutinate either *B. typhosus* or *Ps. protea,* with one exception. The exception was case No. 38, a case of miliary tuberculosis, in which a positive agglutination was obtained with *B. typhosus* in a dilution of 1 : 40; there was no agglutination with *Ps. protea.* This patient gave a history of an attack of typhoid fever three years previously.

SERUM OF NORMAL PERSONS.

To control the results obtained with the serum of cases of typhoid fever, serum was obtained from ten normal persons, and tested for agglutination of the strain of *Ps. protea* (No. 368), used in the majority of the above tests. The first series of 5 sera were tested only in a dilution of 1 : 40. There was no agglutination after several hours. The second series of 5 sera were tested in dilutions of 1 : 10, 1 : 20, and 1 : 40.

TABLE NO. 4.—*Agglutination of Ps. protea (No. 368), agar culture, suspended in salt solution, by the serum of normal persons.*

[+ = Agglutination. ± = Doubtful agglutination. 0 = No agglutination.]

Serum from—	Serum dilutions.								
	1 : 10.			1 : 20.			1 : 40.		
	1 hour.	2 hours.	20 hours.	1 hour.	2 hours.	20 hours.	1 hour.	2 hours.	20 hours.
G	±	+	+	0	+	+	0	0	+
H	+	+	+	0	±	+	0	0	+
CL	+	+	+	0	0	+	0	0	+
LL	±	+	+	0	0	+	0	0	+
C	+	+	+	?	+	+	0	0	+

From these tests it appears that *Ps. protea* is agglutinated by normal human serum in dilution of 1 : 10 within one hour; in dilution of 1 : 20, sometimes within two hours; in dilution of 1 : 40 only after two to twenty hours. The agglutinations with the serum of typhoid fever cases, therefore, in dilutions of 1 : 40 or over, taking place within one hour, can not be regarded as due to the normal agglutinin of human serum.

SERUM OF PERSONS VACCINATED WITH B. TYPHOSUS.

Specimens of serum from three persons vaccinated with *B. typhosus* were obtained through the courtesy of Surg. C. S. Butler, U. S. Navy, and Maj. Frederick Russell, U. S. Army. The results obtained with these specimens are as follows:

	Maximum dilution in which serum agglutinates.	
	B. typhosus.	Ps. protea, 368.
Specimen No. 1, serum of man vaccinated with B. typhosus about three months previously	Negative 1 : 40	± 1 : 40.
Specimen No. 2, date of vaccination unknown	+1 : 100	± 1 : 50.
Specimen No. 3, date of vaccination unknown	+1 : 100	Negative, 1 : 50.

These tests are considered unsatisfactory by reason of the low agglutinating power of the sera for *B. typhosus.* They serve, however, to indicate that the agglutinin for *Ps. protea* may be increased in the serum of a person by the reaction to a known pure culture of *B. typhosus,* and are to that extent evidence against *Ps. protea* agglutinin in the serum of cases of typhoid fever being the result of a mixed intestinal infection.

ABSORPTION OF AGGLUTINS FROM THE SERUM OF TYPHOID FEVER CASES BY SATURATION WITH B. TYPHOSUS AND PS. PROTEA.

It was practicable to carry out the absorption of agglutinins by saturation in only two cases.

The first experiment was with the serum of case No. 51. A 1 : 10 dilution of this serum was made with physiologic salt solution, and divided into two equal portions. The first portion was saturated with typhoid bacilli by adding a large amount of agar culture of *B. typhosus,* scraped gently from the surface of agar slants with an oese and rubbed up in the serum dilution. This was allowed to stand over night at 37° C. and then centrifuged until clear. The second portion of 1 : 10 serum dilution, without the addition of any culture, was kept under the same temperature conditions as a control. Both portions were then tested in various dilutions against suspensions, in physiologic salt solution, of twenty-four hour agar cultures of *B. typhosus* and *Ps. protea.* Observations were recorded at the end of one and two hours at 37° C.

Table No. 5.—*Agglutination of B. typhosus and Ps. protea No. 368, by serum of case No. 51, before and after saturation with B. typhosus.*

[+ = Distinct agglutination. ± = Partial agglutination. 0 = No agglutination. — = No test made. * = Limit of agglutination before saturation.]

Serum dilutions	1 : 10		1 : 20		1 : 40		1 : 80		1 : 160		1 : 320		1 : 640	
Time of observation......hours	1	3	1	3	1	3	1	3	1	3	1	3	1	3
	Control (unsaturated serum).													
B. typhosus	±	+	+	+	0	0	—	—	—	—	—	—	—	—
Ps. protea, No. 368	—	—	+	+	+	+	+	+	+	+	0	±	0	0
	After saturation with B. typhosus.													
B. typhosus	0	0	0	0*	—	—	—	—	—	—	—	—	—	—
Ps. protea, No. 368	—	—	+	+	0	0	0	0	0	0	—	—*	—	—

Although this serum originally agglutinated *Ps. protea* in dilution of 1 : 320, and *B. typhosus* only in dilution of 1 : 20, saturation with *B. typhosus* almost completely removed all agglutinins for *Ps. protea.*

The second absorption experiment was made with the serum of case No. 43, specimen obtained October 2. The serum was diluted 1 : 20 with physiologic salt solution and divided into three equal portions, A, B, and C. The portions B and C were saturated with agar cultures of *B. typhosus* and *Ps. protea,* No. 368, respectively. The technic employed was similar to that above described. Portion A was kept under the same temperature conditions as a control.

Table No. 6.—*Agglutination of B. typhosus and Ps. protea No. 368 by the serum of case No. 43, before and after saturation with B. typhosus and Ps. protea, respectively.*

[+ = Distinct agglutination. ± = Partial agglutination. 0 = No agglutination. — = No test made. * = Limit of agglutination before absorption.]

Serum dilutions	1 : 20		1 : 40		1 : 80		1 : 160		1 : 320		1 : 640		1 : 280	
Time of observation......hours	1	3	1	3	1	3	1	3	1	3	1	3	1	3
	Portion A, control (unsaturated).													
B. typhosus	+	+	+	+	+	+	+	+	+	+	0	+	0	0
Ps. protea, No. 368	+	+	+	+	+	+	+	+	±	±	0	.	—	—
	Portion B, saturated with B. typhosus.													
B. typhosus	0	0	0	0	—	—	—	—	—	—	—	—*	—	—
Ps. protea, No. 368	0	±	0	0	—	—	—	—	—	—*	—	—	—	—
	Portion C, saturated with Ps. protea.													
B. typhosus	+	+	+	+	+	+	+	+	.	+	0	0*	—	—
Ps. protea, No. 368	0	0	0	0	—	—	—	—	—	—*	—	—	—	—

B. typhosus in this case, as in the first case, removed from the serum its own agglutinins, together with all of the agglutinins for *Ps. protea;* while *Ps. protea* removed only its own agglutinins, affecting but slightly the agglutinins of *B. typhosus*. The obvious inference from these two experiments is that the agglutinin affecting *Ps. protea* is a constituent part of the agglutinin for *B. typhosus;* and that the agglutination of *Ps. protea* is to be considered as a *partial* or group agglutination, caused by the combination of some of the receptors of the typhoid agglutinin with bacterial receptors common to *Ps. protea* and *B. typhosus*, rather than a heterologous agglutination. The evidence that it is the specific typhoid antibody which effects the agglutination of *Ps. protea* may be summed up in the following:

1. The constancy of the agglutination of *Ps. protea* by the serum of typhoid fever patients as contrasted with its much slighter agglutination by the serum of controls (serum of normal persons and persons having diseases other than typhoid and paratyphoid fevers).
2. The removal from serum which agglutinates both *Ps. protea* and *B. typhosus*, of *Ps. protea* agglutinins by saturation with *B. typhosus*, and the failure of *Ps. protea* to remove the agglutinins for *B. typhosus*.

Case No. 51 especially illustrates that the physical phenomenon of agglutination is not necessarily an index of the amount of agglutinin which has combined with the bacterial substance. Thus the serum of case No. 51 agglutinated *B. typhosus* only in the dilution of 1: 20, while it agglutinated *Ps. protea* in a dilution of 1: 160. Saturation, however, with *B. typhosus*, showed that the latter was capable of combining with and removing the substance which agglutinated *Ps. protea* so markedly. It was unfortunately impossible, on account of the small amount of serum available, to carry out the saturation of this serum with *Ps. protea*. The results with the serum of case No. 43, however, as well as the similar results obtained with specific animal typhoid immune-serum, warrant the inference that in this case, as in the others, *Ps. protea* would not have removed the typhoid agglutinin. The actual amount of chemical combination of the agglutinin receptors of the serum was certainly as great, and probably greater, with *B. typhosus* than with *Ps. protea*, although the agglutination produced was more marked in the case of *Ps. protea*.

An hypothesis which is suggested in explanation of this observation is as follows:

Assume that the complex specific agglutinin for *B. typhosus* consists of receptors of various kinds, *a*, *b*, *c*, *d*, having combining affinities for corresponding receptors of the *B. typhosus*, A. B, C, D, respectively. Assume further that each *B. typhosus* has numerous receptors of each variety—say, for the sake of simplicity, four of each kind. Then the total receptors of *B. typhosus* may be represented by

AAAA BBBB CCCC DDDD.

It may be assumed now that in order to effect agglutination of *B. typhosus* a certain minimum number of these receptors must be combined with corresponding agglutinin receptors—say, at least 12 receptors.

Thus,

A*a* A*a* A*a* B*b* B*b* B*b* C*c* C*c* C*c* D*d* D*d* D*d*,

or,

A*a*A*a*A*a*A*a* B*b*B*b*B*b*B*b* C*c*C*c*C*c*C*c*,

is sufficient to cause agglutination, while, for instance, the combination

A*a*A*a* B*b*B*b* C*c*C*c* D*d*D*d*

is not sufficient to cause agglutination.

Suppose that *Ps. protea* possesses receptors

DD EE FF GG, etc.,

and that to effect the agglutination of this bacillus requires the combination of only one of the D receptors with the corresponding *d* receptor of the specific typhoid agglutinin.

Now it follows from these assumptions that *Ps. protea* will be agglutinated by the specific typhoid agglutinin in higher dilution than *B. typhosus*. For if a given amount of 1 : 20 dilution of the serum in question contains just enough receptors, $n(3a, 3b, 3c, 3d)$, to cause the agglutination of a given number (n) of typhoid bacilli, this same amount of serum dilution will contain a sufficient number of *d* receptors $(3nd)$ to agglutinate three times that number $(3n)$ of *Ps. protea;* therefore the same serum will just agglutinate *Ps. protea* in dilution of 1 : 60.

Saturation of this serum with typhoid bacilli will obviously remove all of the agglutinin receptors for *Ps. protea*. Saturation with *Ps. protea*, however, can only remove the *d* receptors from the specific typhoid agglutinin, which, assuming the number of *a*, *b*, *c*, *d* receptors in the serum to be equal, would reduce the agglutinating power of this serum for *B. typhosus* by exactly one-fourth.

It is not necessary, however, to assume that in any given serum the relative number of *a*, *b*, *c*, and *d* receptors is equal. On the other hand, it is much more reasonable to assume that they may show wide variations in their relative numbers in different sera, which may account for the different relative agglutinability of identical strains of *B. typhosus* and *Ps. protea*, respectively, by different typhoid immune-sera. It may readily be assumed, moreover, that different strains of *B. typhosus* possess different relative amounts of all receptors A, B, C, and D; also that the chemical affinities A*a*, B*b*, C*c*, and D*d* are not necessarily equal in intensity. These variable factors seem sufficient to explain the variations in agglutinability of different strains of bacteria by the same specific immune-serum, as well as of the same strain of bacteria by different immune-sera.

AGGLUTINATION OF B. TYPHOSUS AND PS. PROTEA BY THE NORMAL SERUM OF LOWER ANIMALS.

The following tests have been made with normal rabbit and horse serum:

TABLE No. 7.—*Agglutination of B. typhosus and Ps. protea by the serum of normal animals.*

[+ = Agglutination. ± = Partial agglutination. 0 = No agglutination. — = No test made.]

Serum dilution.		1:10		1:20		1:40		1:100		1:200		1:400	
Time of observation	hours..	1	3	1	3	1	3	1	3	1	3	1	3
Normal serum, from	Organism:												
Rabbit No. 1...	Ps. protea No. 362[1]..	0	0	—	—	—	—	—	—	—	—	—	—
Rabbit No. 2.........	B. typhosus.........	—	—	0	0	—	—	—	—	—	—	—	—
	Ps. protea No. 366 ..	—	—	0	0	—	—	—	—	—	—	—	—
Rabbit No. 3.........	B. typhosus.........	±	+	±	±	0	0	—	—	—	—	—	—
	Ps. protea No. 368 ..	±	+	+	+	0	0	—	—	—	—	—	—
Rabbit No. 4.........	B. typhosus.........	±	±	0	±	0	0	—	—	—	—	—	—
	Ps. protea...........	+	+	0	0	0	0	—	—	—	—	—	—
Rabbit No. 5......	B. typhosus.........	—	—	0	0	—	—	—	—	—	—	—	—
	Ps. protea...........	—	—	0	0	—	—	—	—	—	—	—	—
Rabbit No. 6.........	B. typhosus.........	—	—	0	0	—	—	—	—	—	—	—	—
	Ps. protea...........	—	—	0	0	—	—	—	—	—	—	—	—
Horse "Frank"......	B. typhosus.........	+	+	±	+	0	+	0	0	—	—	—	—
	Ps. protea...........	+	+	+	+	+	+	±	+	0	0	0	0
Horse, Nat'l No. 37...	B. typhosus.........	+	+	+	+	0	0	0	0	—	—	—	—
	Ps. protea...........	+	+	+	+	+	+	±	+	?	?	0	0

These tests, taken in connection with the tests made with normal human serum, tend to show that *Ps. protea* is in general more readily agglutinated than is *B. typhosus* by the agglutinin present in normal human and horse serum.

In order to determine whether in normal serum the same substance effects the agglutination of both *Ps. protea* and *B. typhosus*, an absorption experiment was carried out with the normal serum of horse "Frank." The serum, diluted 1 : 5 with physiologic salt solution, was divided into three equal parts, A, B, and C. B and C were saturated with agar cultures of *B. typhosus* and *Ps. protea* No. 368, respectively, at 37° C., while A was kept under the same temperature conditions as a control. B and C were centrifuged clear after repeated saturation, and all three portions tested for the agglutination of *B. typhosus* and *Ps. protea* No. 368.

TABLE No. 8.—*Agglutination of B. typhosus and Ps. protea by normal horse serum, Frank, before and after saturation.*

[+=Agglutination. ±=Partial agglutination. 0=No agglutination. —=No test made. **=Limit of agglutination before saturation.]

Serum dilutions	1 : 5		1 : 10		1 : 20		1 : 40		1 : 100		1 : 200	
Time of observation.....hours	1	3	1	3	1	3	1	3	1	3	1	3
	Portion A, Control (unsaturated).											
B. typhosus	+	+	+	+	±	±	0	?	0	0	—	—
Ps. protea, No. 368	+	+	+	+	+	+	±	+	±	+	±	+
	Portion B, after saturation with B. typhosus.											
B. typhosus	0	0	0	0	0	0**	0	0	0	0	—	—
Ps. protea, No. 368	+	+	+	+	±	+	0	+	0	±	—	—**
	Portion C, after saturation with Ps. protea, No. 368.											
B. typhosus	+	+	±	+	0	±**	0	0	0	0	—	—
Ps. protea, No. 368	?	?	0	0	0	0	0	0	0	0	—	—**

Temperature 37° C. for one hour; room temperature for remainder of three hours.

From this it appears that in normal horse serum the agglutinins of *B. typhosus* and *Ps. protea* are independent (or heterologous), which agrees with the observations of Bordet[13] and of Hetsch and Lentz[15] regarding the various agglutinins of normal serum.

SERUM OF ANIMALS IMMUNIZED AGAINST B. TYPHOSUS.

The stock typhoid-agglutinating serum already referred to as used in routine examinations in the Hygienic Laboratory is a horse serum obtained from Parke, Davis & Co., which agglutinates the laboratory stock culture of *B. typhosus* in a dilution of 1:20,000, and has on some occasions given a partial reaction in a dilution of 1:50,000. The agglutinating strength of this serum for *Ps. protea*, strains Nos. 362^t, 368, etc., has been repeatedly determined, and found to be 1:1,000 to 1:2,000, varying to this extent with different strains, and with the same strain under different conditions of culture. This serum was recently carefully tested against the following cultures of *Ps. protea* from the Washington tap water; Nos. 362^t, 366, 366^t, 368, 370, 370^t, 372^t, 374^t, 468^B, all of which had been cultivated for more than four months on artificial culture media under nearly identical conditions. All of these strains were strongly agglutinated in dilution of 1:1,000, and distinctly in a dilution of 1:2,000, within an hour at 37° C. Strains Nos. 366^t, 368, 374^t, and 468^B

showed noticeably less strong agglutination than the others. The controls of 368 and 374^1 showed partial spontaneous agglutination, all the other controls remaining perfectly homogeneous over forty-eight hours.

This serum has been used in the Hygienic Laboratory in the identification of a great number of bacteria, and has never been found to agglutinate, in dilutions approaching 1: 1,000, any organism other than *B. typhosus*, except those above mentioned, a strain of Danysz virus, and a few strains of bacilli isolated from water and resembling *B. coli*. Among the identified bacilli which have been tested against this serum and found not to be agglutinated even in a dilution of 1 : 50 are several strains of *B. paratyphosus* A and *B. paratyphosus* B; *B. paracolon* (Cushing); *B. dysenteriæ* (Kruse type); *B. cholerae suis; B. icteroides; B. enteritidis* (Gartner).

Another typhoid-agglutinating horse serum (Smith), which agglutinates *B. typhosus* "Hopkins" in a dilution of about 1: 5,000, agglutinates *Ps. protea* No. 362^1 in a dilution of 1 : 200.

The serum of a rabbit immunized against *B. paratyphosus* B, which agglutinates that organism in a dilution of 1: 1,000, fails to agglutinate *Ps. protea* No. 368 in a dilution of 1: 40.

A rabbit was immunized by repeated intraperitoneal injections of chloroformed agar cultures of *B. typhosus* "Hopkins," in increasing amounts, followed later by injections of live cultures of "Hopkins." The serum of this rabbit was tested at intervals for agglutinins for *B. typhosus* and *Ps. protea* No. 368, as shown in the following table:

TABLE No. 9.—*Agglutination of B. typhosus "Hopkins" and Ps. protea No. 368, by the serum of rabbit No. 3, immunized against B. typhosus "Hopkins" by intraperitoneal injections of agar cultures.*

[+ = Distinct agglutination. ± = Partial agglutination. 0 = No agglutination. — = No test made.]

Period of immunization when serum was taken.	Culture.	Serum dilutions.							
		1 : 20.	1 : 40.	1 : 100.	1 : 500.	1 : 1,000.	1 : 5,000.	1 : 10,000.	1 : 20,000.
Before first injection	B. typhosus	+	+	–	–	–	–	–	–
	Ps. protea, No. 368	+	0	–	–	–	–	–	–
11 days after beginning of immunization	B. typhosus	–	–	+	+	0	–	–	–
	Ps. protea, No. 368	–	–	+	±	0	–	–	–
[illegible] days after beginning of immunization.	B. typhosus	–	–	–	+	+	+	–	+
	Ps. protea, No. 368	–	–	–	+	±	0	–	–
60 days after beginning of immunization.	B. typhosus	–	–	–	–	+	+	+	±
	Ps. protea	–	–	+	+	+	0	–	–

Observed one hour at 37° C.

Rabbit No. 5 was given two intraperitoneal injections of agar cultures of *B. typhosus*, strain "Bentz," heated to 60° C., for twenty minutes. He received at the first injection half of an agar slant culture; at the second injection, two days later, a whole agar slant culture.

TABLE No. 10.—*Agglutination of B. typhosus "Bontz" and Ps. protea No. 370, by serum of rabbit No. 5, after 2 injections of cultures of B. typhosus "Bontz."*

[+=Agglutination. ±=Partial agglutination. 0=No agglutination. —=No test made.]

Serum dilutions		1:20		1:50		1:100		1:200		1:500	
Time of observation	hours	1	3	1	3	1	3	1	3	1	3
Before first injection	B. typhosus, "Bontz"	0	0	—	—	—	—	—	—	—	—
	Ps. protea, No. 370	0	0	—	—	—	—	—	—	—	
2 days after first injection	B. typhosus, "Bontz"	0	0	0	0	0	0	—	—	—	—
	Ps. protea, No. 370	0	+	0	0	0	0	—	—	—	—
4 days after first injection	B. typhosus, "Bontz"	0	0	0	0	0	0	—		—	—
	Ps. protea, No. 370	0	+	0	±	0	0	—	—	—	—
6 days after first injection	B. typhosus, "Bontz"	+	+	+	+	+	+	0	+	0	0
	Ps. protea, No. 370	+	+	+	+	+	+	0	±	0	0
12 days after first injection	B. typhosus, "Bontz"	—	—	—	—	+	+	+	+	0	±
	Ps. protea, No. 370	—	—	—	—	+	+	±	±	0	0
17 days after first injection	B. typhosus, "Bontz"	—	—	—	—	+	+	+	+	0	±
	Ps. protea, No. 370	—	—	—	—	+	+	0	0	0	0

In the case of rabbit No. 5, the only case where observations were made sufficiently early, it was found that the serum developed the property of agglutinating *Ps. protea* before it showed agglutinins for *B. typhosus*. In both cases (rabbits Nos. 3 and 5) the limit of agglutination for *Ps. protea* approximated quite closely the limit for *B. typhosus* in the early stages of immunization, while later the serum agglutinated *B. typhosus* in considerably higher dilutions than *Ps. protea*. The charts following show this fact more strikingly than the figures already given.

CHART NO. 3.

Showing development of agglutinins for *B. typhosus* and for *Ps. protea* in serum of rabbit No. 3 in course of immunization against *B. typhosus* "Hopkins."

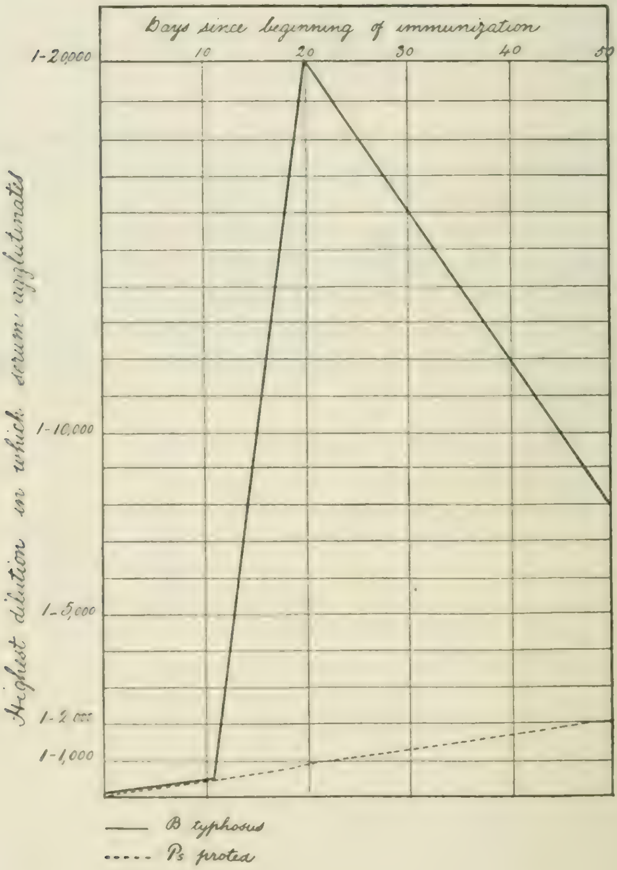
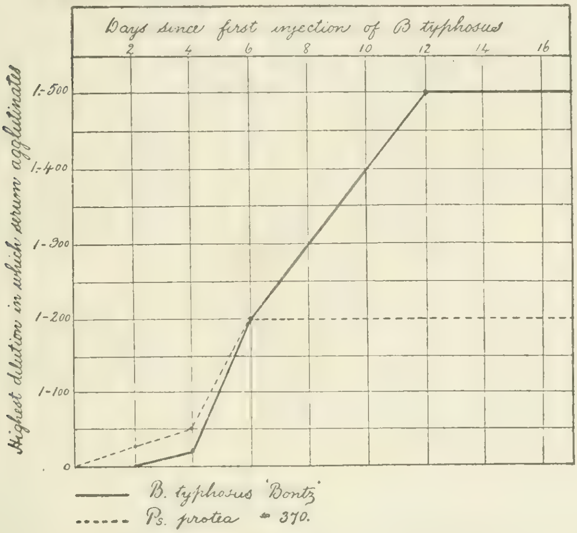


CHART NO. 4

Showing development of agglutinins for *B. typhosus* "Bontz" and *Ps. protea* in serum of rabbit No. 5 after two injections of *B. typhosus* "Bontz."



ABSORPTION OF AGGLUTININS FROM TYPHOID-AGGLUTINATING HORSE-SERUM.

To determine whether the agglutination of *Ps. protea* by animal typhoid-immune serum is a partial agglutination effected by the specific typhoid agglutinin, as already demonstrated to be the case with human serum from cases of typhoid fever, an attempt was made to remove the agglutinins from the specific typhoid-agglutinating horse serum (Parke, Davis & Co.) already mentioned. Equal portions of this serum, diluted 1 :50, were saturated with agar cultures of *B. typhosus* and *Ps. protea* No. 368, respectively, while a third portion of the same dilution was kept as a control. The following table embraces the results obtained:

TABLE No. 11. *Agglutination of B. typhosus and B. proteus No. 368* by specific typhoid immune horse serum (Parke, Davis & Co.).*

[+ = Distinct agglutination. ± = Partial agglutination. 0 = No agglutination. — = No test made. * = Limit of agglutination before saturation.]

Serum dilutions.	1 : 50.		1 : 100.		1 : 200.		1 : 500.		1 : 1,000.	
Time of observation.............hours.	1	20	1	20	1	20	1	20	1	20
	Control; serum not saturated.									
B. typhosus	+	+	+	[illegible]	[illegible]	+	+	[illegible]	+	+
Ps. protea	+	+	+	[illegible]	[illegible]	+	+	+	+	+
	Same serum after saturation with B. typhosus.									
B. typhosus	±	+	0	±	0	0	0	0	0	0
Ps. protea	+	+	+	+	±	±	0	±	0	0
	Same serum after saturation with Ps. protea No. 368.									
B. typhosus	+	+	—	—	—	+	—	[illegible]	[illegible]	[illegible]
Ps. protea	+	[illegible]	[illegible]	[illegible]	0	[illegible]	0	[illegible]	0	0

TABLE NO. 11.—*Agglutination of B. typhosus and B. proteus No. 368 by specific typhoid immune horse serum (Parke, Davis & Co.)*—Continued.

Serum dilutions.	1 : 2,000.		1 : 5,000.		1 : 10,000.		1 : 20,000.		1 : 40,000.	
Time of observation .... hours..	1	20	1	20	1	20	1	20	1	20
	Control; serum not saturated.									
B. typhosus	+	+	+	+	+	+	+	+	+	+
Ps. protea	±	±	0	0	–	–	–	–	–	–
	Same serum after saturation with B. typhosus.									
B. typhosus	0	0	0	0	–	–	–	–	–	–*
Ps. protea	0	0*	0	0	–	–	–	–	–	–
	Same serum after saturation with Ps. protea, No. 368.									
B. typhosus	+	+	+	+	+	+	+	+	+	+*
Ps. protea	0	0*	0	0	–	–	–	–	–	–

The results of a similar experiment with the specific typhoid agglutinating serum of rabbit No. 3 are given in the table following. This serum was also tested, before and after saturation, against Danysz virus, to determine whether the same group agglutinins affected both *Ps. protea* and Danysz virus:

TABLE NO. 12.—*Agglutination of B. typhosus "Hopkins," Ps. protea No. 370, and B. danysz virus, by serum of rabbit No. 3, immunized against B. typhosus "Hopkins."*

[+ = Agglutination. ± = Partial agglutination. 0 = No agglutination. – = No test made. * = Limit of agglutination before saturation.]

Serum dilutions...	1 : 25.	1 : 50.	1 : 100.	1 : 500.	1 : 1,000.	1 : 2,000.	1 : 5,000.	1 : 10,000.	1 : 20,000.
	Control, serum unsaturated.								
B. typhosus "Hopkins"	+	+	+	+	+	+	+	+	0
Ps. protea, No. 370	+	+	+	+	+	0	–	–	–
B. Danysz virus	+	+	+	+	+	0	–	–	–
	Same serum, saturated with B. typhosus "Hopkins."								
B. typhosus "Hopkins"	+	±	0	0	0	0	0	0*	0
Ps. protea, No. 370	0	0	0	0	0*	–	–	–	–
B. Danysz virus	–	0	0	0	0*	–	–	–	–
	Same serum saturated with Ps. protea, No. 370.								
B. typhosus "Hopkins"	+	+	+	+	+	+	+	+*	–
Ps. protea, No. 370	0	0	0	0	0*	0	–	–	–
B. Danysz virus	–	–	–	–	+*	0	–	–	–

The results of the experiments with the rabbit serum agree completely with the results obtained by saturation of the serum of cases of typhoid fever Nos. 43 and 51. *B. typhosus*, in removing its own specific agglutinin, removes also the specific agglutinin for *Ps. protea*, while *Ps. protea* removes only its own agglutinins, without appreciably diminishing the specific typhoid agglutinin. The agglutinins affecting *Ps. protea* and *B. Danysz* virus, while they are both "group" agglutinins, bound to the specific typhoid agglutinin, are independent of each other, as *Ps. protea* does not remove nor diminish the agglutinin for *B. Danysz* virus.

It was found impossible to remove from the highly potent horse serum all the agglutinins for either *B. typhosus* or *Ps. protea;* still, the results are of practically the same significance.

AGGLUTINATION OF B. TYPHOSUS AND PS. PROTEA BY THE SERUM OF ANIMALS IMMUNIZED AGAINST PS. PROTEA.

Two rabbits have been immunized against *Ps. protea* Nos. 362^t and 366, respectively, to determine the development of the specific agglutinins for these organisms and the development of partial agglutinins for *B. typhosus* and other bacteria.

Rabbit No. 1 was immunized by intraperitoneal injections of 24-hour bouillon cultures of *Ps. protea* No. 362,^{t} in increasing doses. The cultures were at first killed by exposure to chloroform, but later live cultures were used. The development of the agglutinins for *Ps. protea* No. 362^t and other identical strains of *Ps. protea* isolated by the same technic from the filtered water, was as follows:

Before immunization, serum failed to agglutinate *Ps. protea* No. 362^t in a dilution of 1 : 10.

Eleven days after the beginning of immunization, the serum agglutinated *Ps. protea* No. 362^t (bouillon culture), in a dilution of 1 : 200 +.

Nineteen days after the beginning of immunization, the serum agglutinated *Ps. protea* No. 362^t (agar culture), in a dilution of 1 : 1,000 + ; negative in dilution of 1 : 4,000.

Two and one-half months after the beginning of immunization the serum agglutinated *Ps. protea* No. 362^t (agar culture) in a dilution of 1 : 10,000.

Repeated tests were made with *B. typhosus*, which was never agglutinated, even in dilutions of 1 : 40. Uniformly negative results were also obtained in a dilution of 1 : 40 with *B. paratyphosus* A, *B. paratyphosus* B, *B. paracolon* (Cushing), *B. icteroides*, *B. choleræ suis*, *B. cloacæ*, *B. fecalis alkaligenes*, and *B. dysenteriæ* (Kruse). Slight delayed agglutination of *B. dysenteriæ* (Shiga) was obtained with a serum dilution of 1 : 40.

Rabbit No. 2 was given subcutaneous injections of chloroformed bouillon cultures, and later live cultures, of *Ps. protea* No. 366. The serum of this animal, after 60 days, agglutinated *Ps. protea* No. 366 and other identical strains in a dilution of 1 : 400; it did not agglutinate *B. typhosus* in a dilution of 1 : 40.

The possibility was considered that *B. typhosus*, though not agglutinated by this immune-serum, might combine with some of the *Ps. protea* agglutinin. Accordingly, to determine this point, a large amount of agar culture of *B. typhosus* was added to a 1 : 10 dilution of the serum of rabbit No. 1, and kept for several hours at 37° C. After centrifuging until clear, this serum was tested for agglutination with *Ps. protea* No. 368, and found to be fully as active as the control serum, which had not been treated with typhoid bacilli. The specific agglutinin for *Ps. protea*, then, does not combine with *B. typhosus*.

To determine the specificity of the agglutinins produced by *Ps. protea*, tests were made with the serum of rabbit No. 1 against the following more or less closely biologically related organisms:

1. Three stock cultures of *Proteus vulgaris*, fully identified, morphologically and culturally.
2. Five strains of bacilli of the colon group, isolated from water and agglutinated in dilutions of 1 : 500 to 1 : 2,000 by the specific typhoid agglutinating horse serum (Parke, Davis & Co.) previously referred to.
3. Two strains of pseudomonas isolated from the filtered Potomac River water, and differing from *Ps. protea* only in their failure to produce gas in glucose bouillon.
4. A bacillus isolated from well water, culturally identical with 3.
5. A pseudomonas from unfiltered Potomac River water, differing from *Ps. protea* in the formation of gas in saccharose bouillon.
6. Two strains of pseudomonas, Nos. 504 and 540, isolated from filtered water, morphologically and culturally identical with *Ps. protea*. Neither of these cultures is agglutinated by the specific typhoid agglutinating horse serum above mentioned.

None of the above cultures was agglutinated by the specific *Ps. protea* agglutinating serum of rabbit No. 1, even in dilution of 1 : 50, except No. 504, which was agglutinated only in dilution of 1 : 200.

A rabbit (No. 6) was injected intraperitoneally with killed agar cultures of No. 504. The serum from this animal developed agglutinins for No. 504, and for *Ps. protea* No. 370, as shown in the following table:

TABLE NO. 13.—*Agglutination of Ps. No. 504 and Ps. protea No. 370, by the serum of rabbit No. 6, immunized against Ps. No. 504.*

[+ = Agglutination. ± = partial agglutination. 0 = No agglutination. — = No test made.]

	Serum dilutions...	1 : 20		1 : 50		1 : 100		1 : 200		1 : 500		1 : 1,000		1 : 2,000		1 : 5,000	
	Time......hours..	1	24	1	24	1	24	1	24	1	24	1	24	1	24	1	24
Days since beginning of immunization:																	
Before injection..	No. 504....	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Ps. protea No. 370.	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16 days after first injection.	No. 504....	—	—	—	—	+	+	+	+	+	+	+	+	+	+	0	0
	Ps. protea No. 370.	—	—	—	—	+	+	+	+	+	+	+	+	±	±	0	0
[illegible] days after last injection.	No. 504....	—	—	+	+	+	+	—	—	+	+	+	+	+	+	+	+
	Ps. protea No. 370.	—	—	+	+	+	+	—	—	±	±	0	0	0	0	0	0

Serum drawn from this rabbit (No. 6) on March 14 (36 days after the beginning of immunization), was diluted 1:25 with physiological salt solution and divided into three equal portions, A, B, and C; portion B was saturated with No. 504; portion C with *Ps. protea* No. 370, and portion A, unsaturated, kept under the same temperature conditions as a control. Portions A, B, and C were tested for agglutinating properties against No. 504 and *Ps. protea* No. 370, giving the results shown in the table following:

TABLE NO. 14.—*Agglutination of Ps. No. 504 and Ps. protea No. 370 by the serum of rabbit No. 6 (immunized against Ps. No. 504), before and after saturation with Ps. No. 504 and Ps. protea No. 370.*

[+ = Agglutination. ± = Partial agglutination. 0 = No agglutination. — = No test made. * = Limit of agglutination before saturation.]

Serum dilutions	1 : 25		1 : 50		1 : 100		1 : 500		1 : 1,000		1 : 2,500		1 : 5,000	
Time, hours	1	18	1	18	1	18	1	18	1	18	1	18	1	18
Portion A, control, serum before saturation.														
Ps. No. 504	—	—	—	—	+	+	+	+	+	+	+	+	+	+
Ps. protea No. 370	—	—	—	—	+	+	±	±	0	0	0	0	0	0
Portion B, same serum after saturation with Ps. No. 504.[a]														
Ps. No. 504	—	—	+	+	+	+	0	+	0	±	0	±	0	0*
Ps. protea No. 370	—	—	0	0	0	0	0	0*	0	0	0	0	0	0
Portion C, same serum after saturation with Ps. protea No. 370.														
Ps. No. 504	—	—	+	+	+	+	+	+	+	+	+	+	+	+*
Ps. protea No. 370	—	—	0	0	0	0	0	0*	0	0	0	0	0	0

[a] The saturation with Ps. No. 504 seems to have been incomplete.

These results indicate that *Ps. protea* and No. 504 are not identical in their agglutinating properties, but closely related, as each produces group agglutinins for the other. Ps. No. 540 is not agglutinated by the specific agglutinating serum of either No. 504 or *Ps. protea.* Further study is necessary to determine whether these three "strains" can be differentiated by other properties than their different agglutinating and agglutinogenic reactions.

For the present it remains undetermined whether the nine strains of *Ps. protea* which have identical agglutinative as well as cultural characteristics represent a species distinct from Nos. 504 and 540 or simply represent "strains" of the same species whose agglutinating substance has undergone some change incident to certain antecedents in environment. In other words, it is undetermined as yet whether the agglutinative properties of the nine strains of *Ps. protea* studied are constant, specific properties or merely inconstant strain peculiarities.

It seems that some biologic conditions in the filtered Potomac River water during the months of August and September are peculiarly favorable either to the development of the species *Ps. protea*, or to the development in a species constantly present of peculiar agglutinative properties.

SUMMARY.

1. By means of a selective technic, a pseudomonas, provisionally classed as a new species, *Ps. protea*, showing quite constantly the characteristic of being agglutinated by specific typhoid immune-serum, (*a*) has been found in the filtered Potomac River water supplied to Washington, D. C., quite frequently during the month of August, 1909, less frequently during September and October, and not at all during November and December; (*b*) the technic employed has failed to demonstrate this organism in Potomac River water before filtration, in the water of a limited number of springs and wells examined, or in the stools and urine of a large number of persons examined.

2. Nine strains of this organism, isolated at different times, have shown constantly and in approximately the same degree, the property of being agglutinated by specific typhoid immune-serum from persons having typhoid fever and from lower animals immunized against *B. typhosus*. With the serum of cases of typhoid fever in various stages, this organism is agglutinated in a larger percentage of cases than is *B. typhosus*, the difference being especially marked in the early stages of the disease. The serum of cases of typhoid fever quite often agglutinates *Ps. protea* in higher dilutions than it agglutinates *B. typhosus*. A limited number of observations have shown that specific typhoid immune-serum from lower animals, in the early stages of immunization, agglutinates *Ps. protea* in approximately the same dilution as it agglutinates *B. typhosus*, but later, as the agglutinating strength for *B. typhosus* increases, the titer for *Ps. protea* fails to increase proportionately. Highly potent specific typhoid immune-sera agglutinate *Ps. protea* only about one-tenth as actively as they agglutinate *B. typhosus*.

3. The normal serum of man, the horse, and the rabbit agglutinates *Ps. protea* only in low dilutions. So far as studied, the serum of persons having malaria, pneumonia, tuberculosis, and slight febrile disturbances shows no increase in the agglutinins for this organism.

4. The results of absorption experiments indicate that the agglutination of *Ps. protea* by specific typhoid agglutinating serum is effected by combination with a portion of the specific typhoid agglutinin; that it is therefore a "group" agglutinin.

5. Animals injected with cultures of *Ps. protea* develop agglutinins for this organism, but do not develop agglutinins for *B. typhosus* or other organisms of the colon-typhoid group.

6. Further work is necessary to determine whether agglutination by typhoid immune-serum is a characteristic of this species or a "strain" peculiarity dependent upon environmental antecedents.

7. Further work is also necessary to determine whether all strains of *Ps. protea* are capable of causing the production of agglutinins which are specific and distinct for all other strains of the same species.

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III. SOME CONSIDERATIONS ON COLORIMETRY, AND A NEW COLORIMETER

By Norman Roberts

SOME CONSIDERATIONS ON COLORIMETRY, AND A NEW COLORIMETER.[a]

By Norman Roberts,

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During the past two or three years we have had occasion to make a considerable number of colorimetric determinations in the Division of Chemistry of the Hygienic Laboratory, in connection with sanitary water analysis. In this connection, Dr. Joseph H. Kastle, then chief of the division, offered the suggestion that I undertake to design a simple and inexpensive colorimeter, sufficiently accurate for the ordinary colorimetric determinations and sufficiently light and compact for field work in sanitary water analysis. With the funds available for the purpose considerable difficulty was experienced in securing the skilled mechanical labor required for the proper construction of this instrument; hence considerable time has been spent in its construction. As is frequently the case in matters of this kind the problem gradually increased beyond its original scope as the work progressed, and with the aid of the new instrument it was the intention of Doctor Kastle and myself to undertake certain studies in colorimetry. Circumstances prevented, however, and it has therefore been deemed expedient to write up the following description of the instrument, in the hope that it may be found useful to those who have occasion to employ colorimetric methods.

Colorimetric methods have for many years been of great use, especially in commercial analyses, on account of their simplicity, rapidity, and delicacy. Their lack of accuracy alone has stood in the way of their wider application. Contrary to a widespread notion, this lack of accuracy is not inseparable from colorimetry, but is due to crude apparatus and to unscientific methods resulting from a lack of knowledge of the finer details of the chemical reactions involved. Methods and apparatus alike are being progressively improved, and little by little colorimetry is taking its proper place with other exact methods of quantitative analysis.

We were led to devise the colorimeter herein described by reason of the following facts: (1) All colorimeters on the market seem to be unduly complicated and expensive if at all accurate, or if at all cheap to be grossly inaccurate; (2) the good features available are scattered around among a number of instruments instead of all being

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embodied in one; (3) no accurate instrument seems to be available in a portable form for field use.

In the course of our attempts at devising our colorimeter we found that:

1. The chief essential to accuracy is the optical perfection of the glass pieces that limit the columns of liquid above and below, since in order to obtain accurate results the liquid columns must be cylinders or prisms with plane and parallel ends, and the glass end pieces must transmit light without deviation or other optical effect except a possible slight diminution in the intensity of the light, which must be equal on both sides.

2. The simplest way to be able to see simultaneously the two beams of light coming upward through the two columns of liquid is to tilt the two limbs of the colorimeter at an angle to each other and place the eye where the two beams intersect ("direct vision"). By this arrangement, however, the two images are seen at a considerable distance apart. They can be brought side by side by placing at the point of meeting of the beams a suitably designed dihedral mirror. The angle of incidence being equal to the angle of reflection, it is a simple mathematical problem to determine the proper angles of inclination of the mirror so that the beams will pass from the point of meeting side by side horizontally forward to the eye. (See fig. 4.)

3. In order to get the longest liquid column with the smallest quantity of liquid and without complicated parts, a pair of telescoping glass-bottomed tubes (container and plunger) is the best arrangement. To hold the excess of liquid when the column is shortened and to provide for its instant return when the column is again lengthened, without excessive length of tubes, the upper part of the container is much enlarged in diameter. To prevent this enlargement from interfering with the opposite container, the enlargement takes the form of a short wide cylinder, placed eccentrically with reference to the narrow tube of the container, and projecting laterally.[a]

It was thought advisable to design the instrument in two forms, the first embodying the most valuable of the features in the simplest and cheapest manner possible; the second containing all the valuable features (original or borrowed) that were found applicable.

The simplified form (fig. 2) consists of the two liquid containers previously described, mounted at an inclination to each other in chambers bored out in a block of wood; the lower ends of these chambers open through the bottom to admit light from below. The upper end carries a paraffined cork or other suitable stopper through which the plunger slides. In this form the adjustment is made by sliding the plungers up and down; the outer surfaces of the narrow parts of the liquid containers are graduated in centimeters and millimeters, these graduations being exposed to view through windows cut in the front of the block of wood. The scale starts below, with zero at the bottom of the cylinder of liquid; the bottom of the plunger (limiting the top of the column of liquid) indicates the reading on the scale. In using this apparatus it is held in one hand and the adjustments made with the other, a sheet of white paper below serving as a reflector.

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[a] Stammer's colorimeter contains a narrow cylinder with a widened upper part that serves as a reservoir, but the enlargement is concentric and the axes of the two beams of light are parallel, not inclined.

The perfected form of the instrument (fig. 1) consists (1) of a stand carrying a pair of white reflectors below and a dihedral mirror above, and (2) a detachable part corresponding to the simplified apparatus described above, but made somewhat differently and more elaborately. This detachable part can be carried in the pocket or in a case like a field glass and used in field work in the same way as the simplified apparatus, from which it differs in that the plungers are fixed above while the containers are moved up and down. This arrangement necessitates a separate sliding support for each container and an outer jacket to hold the two sliding supports. The plungers are held in place above by a pair of brass caps, each containing a cork bushing for the purpose of adjusting the plungers so as to touch the bottom when the scale reading is zero. The sliding support is a cylinder of brass with two perforated diaphragms so arranged as to hold the liquid container in the proper position. (Fig. 3.) A set screw working in a slot in the outer jacket holds the sliding support at any desired height.

The outer jacket is composed of two brass cylinders, slotted for the set screws of the sliding supports and hinged together at the top in such a way as to permit them to touch throughout their entire length or to swing through more or less of an angle; below they are furnished with legs and set screws to hold the braces which maintain the proper angle of divergence when the instrument is in use. A separate single brace is substituted when the instrument is to be held in the hand during use.

The scale is marked on the outer jacket along the beveled edge of the slot; the zero is above and the indicator is at or near the upper edge of the sliding support, which is opposite zero when the bottoms of the plunger and container are in contact. The upper part of the surface of the sliding support which shows through the slot may be marked with a Vernier scale.

The glass containers were blown by the Whitall-Tatum Company. The plungers were made of ordinary glass tubing carefully selected as to external diameter and thickness of wall. The optically perfect bottoms for containers and plungers were made as follows:

We obtained from a spectacle maker a regular stock blank of spectacle glass,[a] 1 cm. thick and about 5 by 8 cm. in area, which he finished off with flat, parallel, polished faces. To make the plugs (the optically perfect bottoms) we first protected the polished surfaces by pasting over each side, with mucilage, a single sheet of the thinnest toilet paper. A brass tube a little larger in internal diameter than the plug desired was then taken, mounted as a drill, and the plugs bored out, using turpentine and carborundum. The open end of the tube (container or plunger) in which the plug was to fit was then ground out with a steel shaping tool moistened with turpentine and carborundum. The plug was next turned down with turpentine and carborundum to the nearest possible approximation to the correct size and shape and the tube and plug were carefully ground together with the finest washed emery until they fitted exactly, and were then cemented together with a very small quantity of paraffin.[b]

The inside of the plunger is blackened with a dead black varnish[c] to within a couple of millimeters of the top of the bottom plug. One or more wide-aperture diaphrams may be placed inside of the plungers if thought necessary. The upper end of the plunger is closed with a thin glass top, held in place by a narrow metal rim.

The plungers and containers are the same in both modifications of the instrument, except that in the simpler form the plungers are a little longer, so that when the lower end is touching the bottom

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[a] Ordinary colorless spectacle glass of good quality, planed and polished by the methods ordinarily employed in making spectacles, is good enough for this purpose, although not absolutely perfect. Plate (mirror) glass, rod glass, or blown glass of any kind is not.

[b] We have found this method much superior to any method of cementing a glass *plate* to the ground *end* of the liquid container, or of using a metal connection. We have allowed (aqueous) liquids to stand in one of these containers continuously for six months without any sign of the bottom loosening. If it does come out it requires only a few minutes to cement it in place again. At the same time the bottom may be readily and safely loosened at will by whisking a few times through the Bunsen flame.

[c] The following recipe answered our purpose and may be satisfactory to others, although it can undoubtedly be improved:

Alcohol (95 per cent)	c. c.	500
Shellac	grams	70
Lampblack	do	6
Fine chalk	do	3

Better results are obtained if the powders contain rather coarse particles, since the surface on drying is rougher and consequently duller. Also, it is not necessary that the varnished plunger be absolutely opaque to light when looked at sidewise, so long as the internal surface appears of a good dead black when the plunger is looked through lengthwise.

enough will project above the rest of the instrument to be easily taken hold of by the thumb and finger for purposes of adjustment.

A simple eyepiece (a weak converging lens, not shown in the figure) may be mounted in front of the dihedral mirror. If the mirror be swung out of the way the instrument may be used by "direct vision," the eye looking directly down the tubes.

The white reflectors beneath may be of porcelain, but are best made of paper, frequently renewed, and held down flat by large metal covers each with a large aperture in the center.

The stand is of wood or metal (preferably brass below and aluminum above), the base being weighted with lead if desired. The detachable part is set astride of the upper edge of the stand, and the legs adjusted at the necessary angle by the braces. The apparatus may be constructed with the dihedral mirror permanently fixed in position (it is represented as adjustable), since the angle of inclination of the limbs, the dihedral angle of the mirror, and the angle formed by the edge of the dihedral mirror with the horizontal, are all interdependent, and independent of anything else.

The mathematical relations between these angles are as follows:

Let ϕ=angle of deviation of leg from vertical;

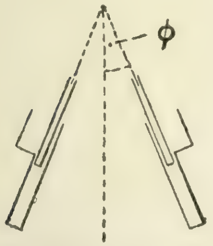
θ=angle of deviation of each wing of mirror from 180° ($=\frac{1}{2}$ supplement of dihedral angle);

γ=angle of inclination of edge of dihedral angle to the horizontal;

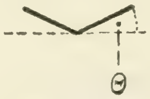
Then $\text{Sin } \theta = 0.707107 \sin \phi$ $(0.707107 = \sin 45°)$.

$\text{Cot } \gamma = \cos \phi$.

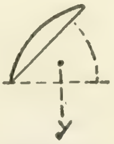
(Lack of space forbids a demonstration of the derivation of these functions.)



Containers and plungers.



Mirror, cross section.



Mirror, side elevation.

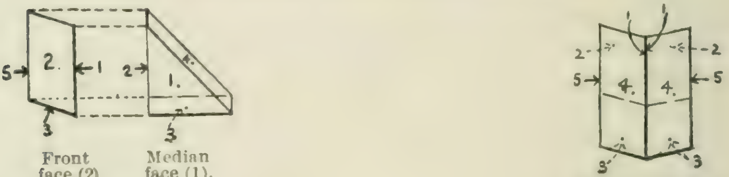
Thus if $\phi=10°$, θ will = about 7°, and γ about 45.5°. Extreme accuracy is not essential in these calculations; nor is it possible, within reasonable limits of cost, to get the angles of the mirror set with less than 30 minutes or so of error.

The dihedral mirror is made of glass semicircles about 2 cm. in diameter and 0.2 mm. thick, silvered on the back and mounted (with a cement composed of boiled linseed oil and red lead) on a block of wood having carefully planed faces at the proper angle. These semicircles must be of ground and polished (not blown) glass.

A somewhat better result (at a much greater cost) is obtained by replacing the silvered semicircles by a pair of internal-reflection prisms in which the entering and emerging rays are perpendicular to the surfaces which they respectively encounter, and the internal-reflecting surface is set at the same angle as are the silvered glasses in the simpler form of mirror. This internal-reflection apparatus (see accompanying diagram) is very hard to describe without the aid of a model. It consists of two symmetrical halves each having the following plane faces:

1. A vertical face which comes in contact with the corresponding face of the other prism in the median line.
2. A vertical face which looks directly forward, forming a continuous surface across the median line with the corresponding face of the other half.

Views of glass internal-reflection blocks perpendicular to—

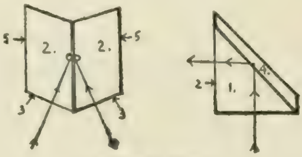


Front face (2)

Median face (1).

Edge of angle between No. 4 faces.

3. A face which looks downward and slightly outward and is perpendicular to the beam of light coming up through the liquid; this face forms with the corresponding face of the other half an obtuse dihedral angle with the edge horizontal and front-to-back.
4. A face from the internal surface of which the light is reflected; this face likewise forms with the corresponding face of the other half an obtuse dihedral angle, the edge of which is inclined at an angle (γ) something over 45° to the horizontal in a vertical median plane.
5. Another face necessary to determine the solid, but not necessary to the optical working of the apparatus, and best made parallel to face 1.

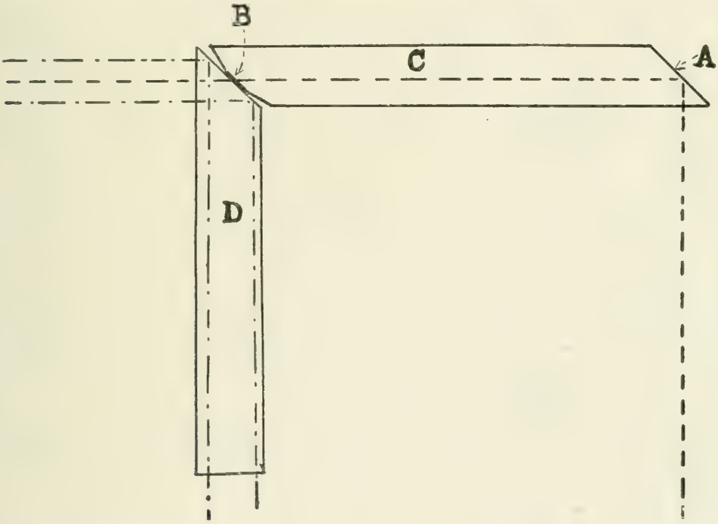


Paths of light.

Thus on each side the beam of light coming upward from below passes through the colored liquid, through face 3 at a right angle (to avoid refraction and dispersion), undergoes total internal reflection by face 4, passes horizontally forward parallel to and tangent with face 1, and emerges perpendicularly through face 2 on each side as a cylinder of light in close contact with the corresponding cylinder of light transmitted through the other half of the prism. Entering the eye these twin cylinders of light appear as tangent circles in the shape of a figure 8 lying on its side. (Fig. 4.)

Other methods (see fig. 5) can be used for securing this juxtaposition of the images; these methods, however, necessitate more or less rearrangement of the apparatus. Thus by the use of the familiar double rhombic, doubly internal-reflecting prisms as used in the Duboscq and other instruments, an instrument resembling both the Duboscq and the Stammer colorimeters (fig. 4) would result, which would seem to be better than either. Polarizing or spectroscopic appliances such as those used with the instruments of Krüss [a] may also be placed in the path of the emerging beams of light. The Lummer-Brodhun prism can be applied to this instrument with great advantage, but the limbs of the instrument would have to be parallel, and the light emerging from above would be thrown horizontally to one side, as is done in the instrument of Smeaton [b] and others.

We have designed (but owing to the difficulty of getting perfect glass we have not yet been able to execute) an improved modification of the Lummer-Brodhun prism for use with this (or other) form of colorimeter, the light coming from below in two parallel paths a short distance apart. The accompanying diagram is almost self-explanatory. A is an internally reflecting surface at 45°. B is the area of contact between the two prisms; the light passes through B in straight lines without loss in any direction, but on striking any other oblique internal surface it is reflected. The right-hand beam of light coming from below is reflected to the left by A, and either passes through B or is scattered by the curved surface of the rest of this face of the horizontal prism C. The left-hand beam of light coming upward through D either passes on upward through B, or is reflected



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[a] Kolorimetrie und Quantitativ Spektranalyse in ihre Anwendung in der Chemie; Voss, Leipzig, 1891.

[b] Journal of the American Chemical Society, 1906, vol. 28, pp. 1433–1435. See diagram in fig. 4.

to the left by the remainder of the plane surface which forms the top of D. Thus there emerge, from the upper portion of the left-hand face of D, two concentric beams of light, the *inner* of which is the light reflected from A through the contact-area B (and therefore a part of the right-hand beam); and the *outer* of which is the light from the left-hand beam coming up through D and reflected from the part of the upper surface of D surrounding B.

The colorimeter may be made of any length of column desired, provided that the proper proportions are maintained, the chief requirement of which is that the wide upper part of the container shall be of sufficient capacity to hold, with some margin, all of the liquid displaced when the plunger is pushed to the bottom, enough liquid having been introduced in the first place to fill completely the lower narrower portion of the container.[a]

Under some circumstances it may be desirable or convenient to use some substance other than glass for the side walls of the containers and plungers. It is even possible to make the block or sliding support that holds the containers of such shape and material as to serve the double purpose of container and container support. Thus the "simplified" apparatus might be still further simplified by replacing the wooden block and the glass containers by a single piece (for each side) of pressed glass, of cast metal heavily gold plated on the inside, of molded rubber, or of some "composition." Of course the bottoms of the containers must still be of optically perfect glass.

The perfected instrument may be made much more cheaply by using wood instead of brass. This will necessitate some slight alterations in design, especially of the sliding support and outer jacket, which will be rectangular in cross section. The wooden outer jacket, instead of being of two cylinders hinged together, is best made in one piece with a wedge of wood inside separating the two sliding supports. (See fig. 5.)

In the interests of accuracy and convenience, it is to be hoped that Nessler tubes and similar crude devices will in the near future be largely superseded by colorimeters. In the meantime, however, we may as well improve the Nessler tube somewhat. First, they should be marked as to height (they are now usually marked as to capacity only), since it is depth of column and not the amount of liquid present that determines the color of the emerging rays. Second, the bottoms should be of optical glass, ground in as here described, instead of being blown as one piece with the rest of the tube; since no two blown bottoms are at all nearly alike, being concave lenses of very varying degrees of strength and irregularity of curvature, with correspondingly great differences in the amount of light transmitted along the axis of the tubes.

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[a] We use for the plungers, tubing 11 mm. in external diameter. The lower, narrower part of the container is 12 mm. in internal diameter and 11 cm. deep, capacity about 12 c. c., of which 10 c. c. are displaced into the reservoir by the descent of the plunger. The reservoir above is 25 mm. in internal diameter and 40 mm. deep, gross capacity about 17 c. c., of which about 3.5 c. c. is occupied by the plunger, leaving a net capacity of 13.5 c. c. There is thus an excess capacity of 3.5 c. c. in the reservoir.

In testing this colorimeter by practical use, over 1,000 observations were made, and at the same time about 200 were made with the Duboscq colorimeter, an instrument costing about $80, and about the best colorimeter now on the market. These observations indicated that our instrument is nearly or quite as accurate as the Duboscq (even when used without the dihedral mirror, and hence with the images widely separated) except for very short lengths of column, our instrument not having a vernier at that time. On the other hand, on account of the longer column of liquid, our instrument accurately measures color tints too faint to be seen with the Duboscq.

An aspect of colorimetry that needs much more study than it has received is the functioning of the man behind the colorimeter. Indeed, all instruments of this kind are merely accessories to the organ of sight. We experimented with a number of persons who had never before in their lives made a colorimetric estimation or anything of the kind, as well as with colorimetrists of varying degrees of experience. We found only one (doubtful) case of a person so deficient in ability to match shades of color as to render it improbable that he could ever do good work with the colorimeter; hence we can not agree with Schreiner[a] that any considerable proportion of persons are debarred from usefulness in colorimetry for physioligical reasons. Some people are, of course, more favored by nature than others, in this as in other respects; but in colorimetry this superiority apparently consists largely in the knack of getting passable results with inferior instruments, and the nearer a colorimeter approaches perfection the less the differences between the results obtained by observers of different degrees of skill.[b] Practice is rewarded by somewhat lower errors, but especially by ability to work longer without disabling fatigue.

It may be thought that color-blindness, relatively frequent though often unsuspected in men, would interfere with the use of this instrument; on the contrary, however, color-blindness would probably in most cases rather help than hinder one as a colorimetrist. The color-blind are in the great majority of cases blind to one or more *tones* only, while as a physiological compensation their eyes are usually more than ordinarily sensitive to differences in *shade*. In practical chemical colorimetry other circumstances make it easy for even a color-blind to avoid mistakes in tone, and sensitiveness as to shade is the only consideration.

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[a] Schreiner, O.; Journ. Amer. Chem. Soc., 1905, vol. 27, pp. 1192.

[b] Extreme cases (relatively few in number) are of course, not meant to be considered, whether on the one hand the comparatively few disabled for colorimetry by blindness, partial or complete, or by some perhaps unsuspected selective nervous defect, or on the other hand the *very* few of preternatural sensitiveness to minute differences in tone and shade.

Women will probably make better routine colorimetrists than men, as their color perception averages better than men's as to both tone and shade. They are also more efficient at any form of monotonous work requiring rapidity of execution without physical overexertion.

At this stage it is impossible to say what percentage of error may be expected when working with a colorimeter of this type. Out of over 1,000 readings made while we were experimenting with the instrument, only a few can be said to have been made under conditions that were even nearly fair to the colorimeter. Up to the very last of the series of tests, the use of the instrument suggested improvements in it. One series of readings by an experienced observer under fairly favorable conditions gave an error for the entire series of 0.13 per cent, the average error for individual readings in this same series being 1.84 per cent, and the extreme error for a single reading 6.35 per cent. Other series of observations by the same observer under less favorable conditions gave average errors for the series of about 2 per cent, average errors for individual readings of about 4 per cent, and extreme errors for single readings of 15 to 35 per cent. These trials were made with accurately prepared solutions of the same chemical composition, thus of course eliminating possible sources of error from other conditions met with in practical work.

In reading the colorimeter one eye only is used at a time. Most observers make a practice of using one eye exclusively, a practice which is bad in more than one way, for the eye used soon tires and loses a great deal in sensibility; hence only a relatively few accurate readings can be made in any one day. Moreover, this prolonged one-sided use is for most persons a severe strain on both eyes. The usual practice with respect to the unused eye is either to hold it shut or to block it off with a black screen. An eye from which light has for some time been excluded is insensitive to color, and hence it is that observers get the usual erroneous notion that it is impossible to use the eyes alternately. The fact is that the eyes can be used alternately with great benefit both to the eyes themselves and as regards the quantity and quality of results if, instead of a black screen, we use a translucent gray screen or better yet, a screen of a color complementary to that of the solution being observed, the eyes being used alternately. A final adjustment should always be made, however, with the better eye, the other being closed; for one eye is almost invariably superior to the other in the matter of color perception, although for most observers the two used alternately in the manner indicated are better than either used exclusively.

The solutions to be compared should be of as nearly as possible the same color strengths as the standards. For some substances this is absolutely necessary; for example, according to Steiger,[a] manganese salts change in tone as well as in shade by dilution. With other compounds this change in tone may not take place, and it is only because of the difficulty of making accurate readings with columns of markedly unequal length that it is desirable to have the colors approximately the same to begin with. In many cases the liquid-containing cylinder of the colorimeter itself is a convenient apparatus in which to make the necessary dilutions (the narrow limb of the container being graduated), the concentrated solution being introduced up to a certain level and the diluting fluid being run in in successive portions until the colors of the unknown and standard match roughly, as determined by looking through the two containers sideways, and the final accurate comparison of the colors being done by taking the reading in the regular way as previously described, making allowance, of course, for the dilution.

Provision could easily be made for temporarily or permanently replacing the liquid standards by Lovibond's or other colored glasses. This would be particularly advantageous where the liquid standard is unstable or hard to standardize accurately, as, for example, in hemoglobin work. In the same way use could be made of liquid standards in sealed cells with flat optically perfect ends.

In the course of our trials of the new instrument we discovered an unsuspected source of error in colorimetric determinations, namely, *adsorption* of certain coloring matters by the glass. This phenomenon was most marked with anilin dyes, especially with methyl-violet and gentian-violet, and to a less extent with fuchsin. It was more marked when the glass surfaces in contact with the liquid were rough than when they were smooth, and the error could be reduced for a given coloring matter by using one portion of the solution to saturate the glass and then using a second portion for the determination. After using the colorimeter with a solution that stained the glass, it was necessary to scrub vigorously with soap and water to remove the stain.

A number of diagrams are appended to show the points of similarity and of difference between our colorimeter and some more or less closely related forms. In common with the Schreiner instrument, our colorimeter has the inclined telescoping tubes and inclined mirror above; in common with the Stammer, the reservoirs formed by the widening of the upper part of the containers. The eccentric placing of the reservoirs above to allow of the closer placing of the two limbs, the method of fitting in the optical-glass bottoms, and the bringing together of the colored images by means of a single reflection by an inclined dihedral mirror are, so far as accessible literature shows, original, at least as applied to a colorimeter.

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[a] Journ. Amer. Chem. Soc., 1908, vol. 30, p. 215.

Advantages of this colorimeter.—The simpler form of this instrument is much more accurate than any form of colorimeter having blown glass or other imperfect bottoms, and should in the near future be purchasable at a very low price. Indeed, if one were equal to the task of grinding in the bottoms, the instrument could easily be homemade. The more elaborate instrument has the advantage of accuracy and convenience, the greatest obtainable depth of column with a small amount of liquid, and more simple construction (hence probable cheapness) than any other instrument having the same degree of accuracy. In addition, the essential part of the instrument is detachable and portable and can be used for field work.

In designing this instrument we have received indispensable advice and assistance from a great many persons, especially from the scientists and expert mechanicians at the Bureau of Standards. We are greatly indebted to Mr. F. A. Edmonds, a Washington optician, for advice and for tedious and difficult experimental glass grinding under discouraging circumstances, when no one else would undertake the work. We also wish to express our obligation to those who helped us with the experiments with the completed instrument—officers and employees of the Hygienic Laboratory and others.

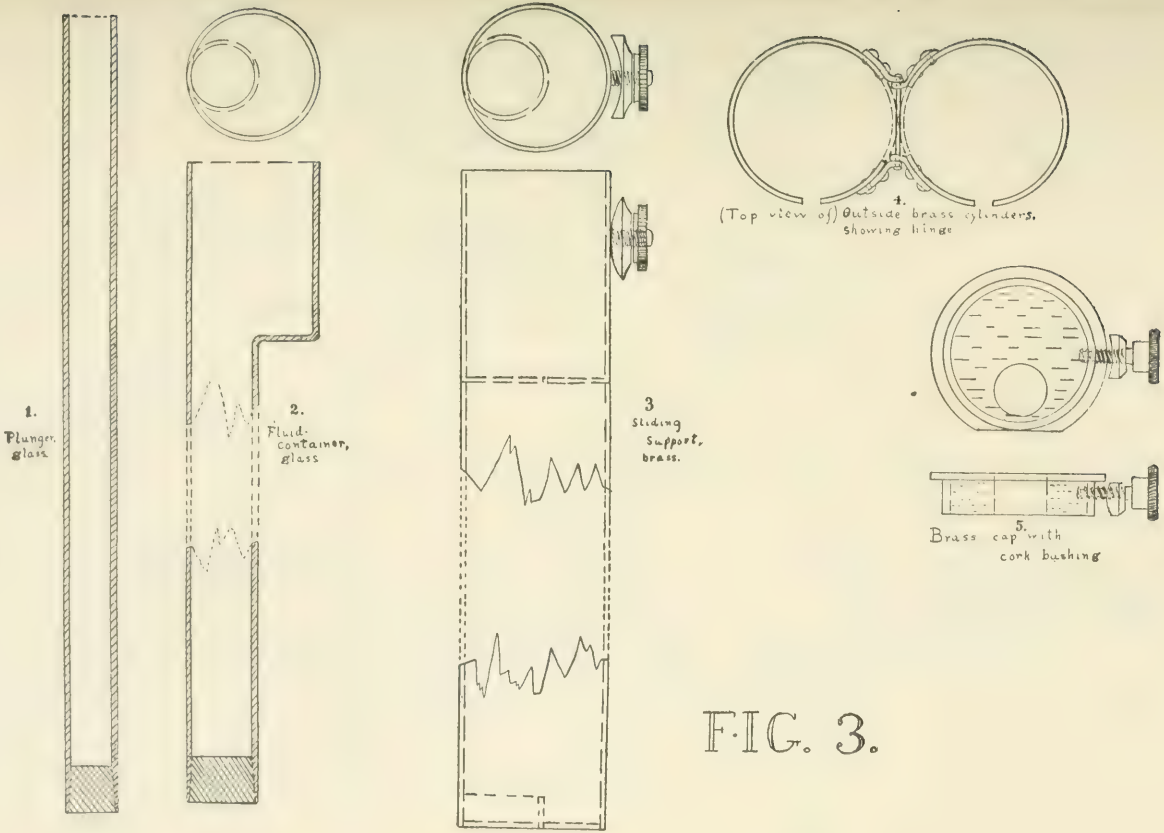

1.
Plunger,
glass
2.
Fluid-
container,
glass
3
Sliding
Support,
brass.
4.
(Top view of) Outside brass cylinders,
showing hinge
5.
Brass cap with
cork bushing

FIG. 3.

Fig. 4.

Various Forms of Immersion-Colorimeter

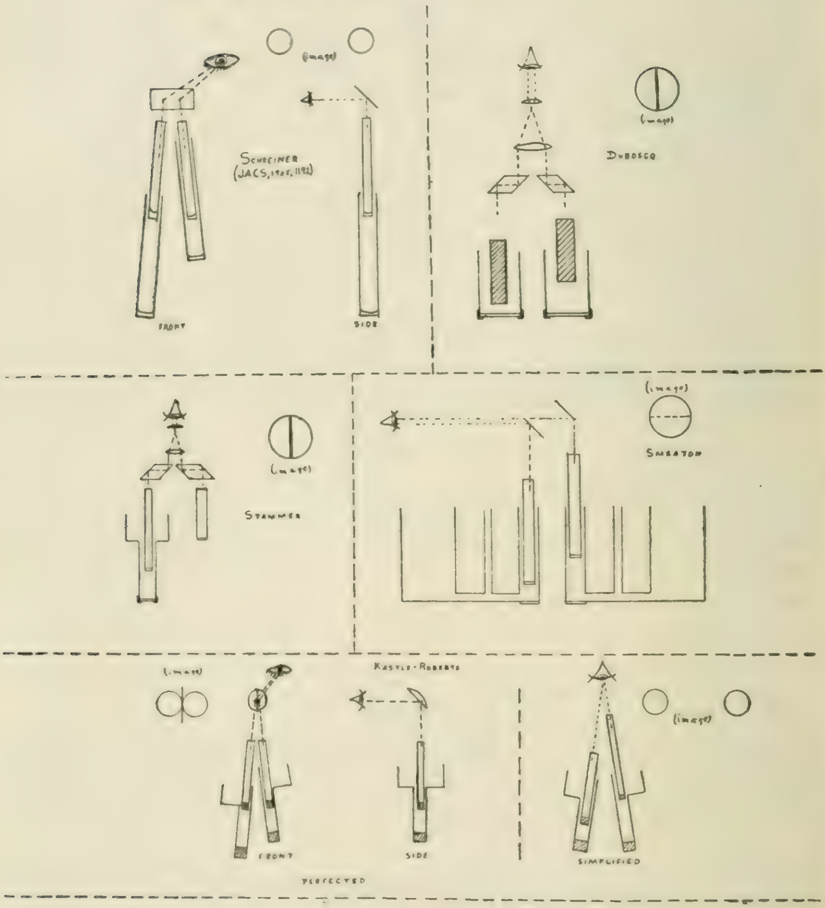
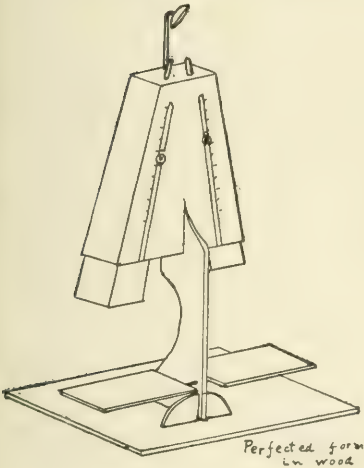
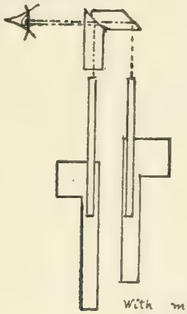


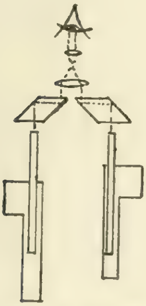
Fig. 5. Possible modifications of K-R Colorimeter.



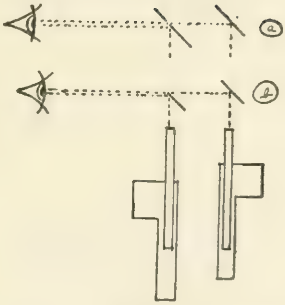
Perfected form, in wood



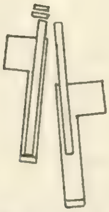
With modified Lummer-Brodhun prism.



With rhombic prisms



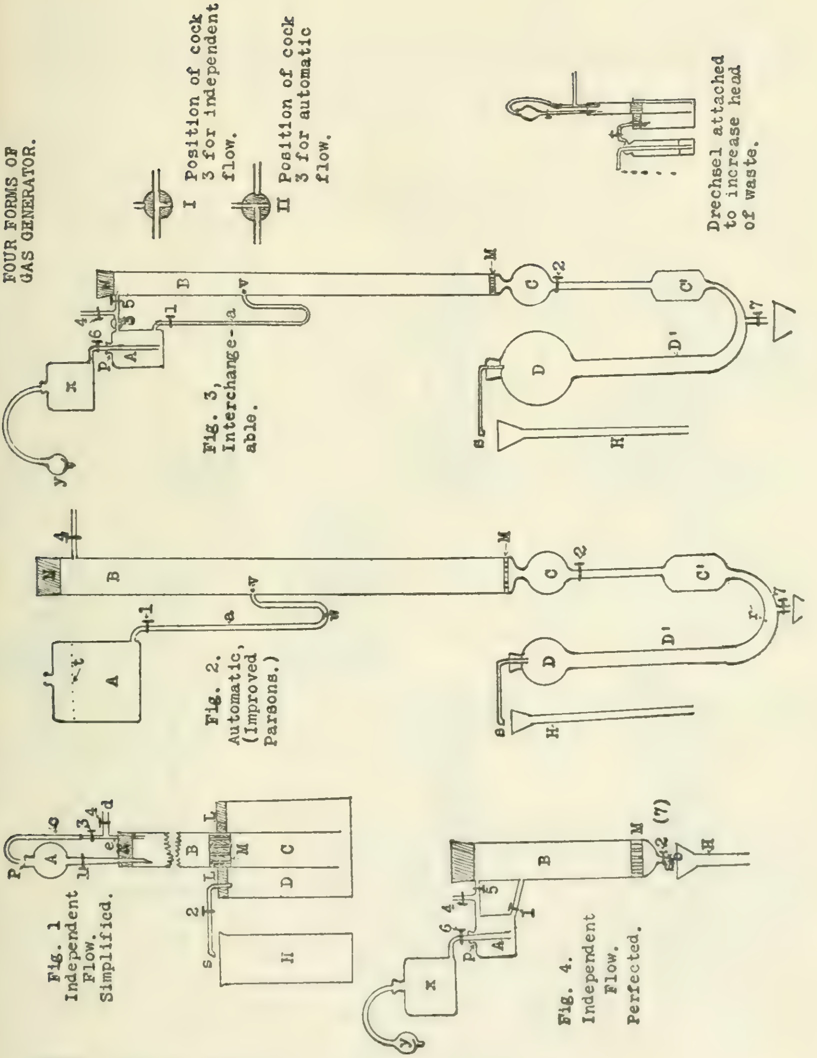
with perforated ⓐ or ⓑ en-echelon mirrors



With standards of colored glass or of liquid in flat cells.

IV. A GAS GENERATOR, IN FOUR FORMS, FOR LABORATORY AND TECHNICAL USE

By Norman Roberts



FOUR FORMS OF GAS GENERATOR.

Fig. 1 Independent Flow. Simplified.

Fig. 2. Automatic, (Improved Parsons.)

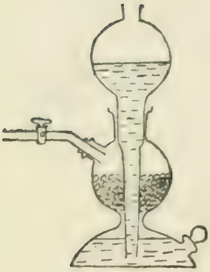
Fig. 3, Interchangeable.

Fig. 4. Independent Flow. Perfected.

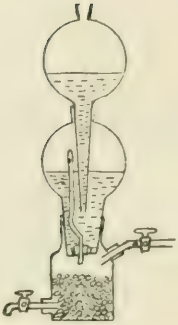
I Position of cock 3 for independent flow.

II Position of cock 3 for automatic flow.

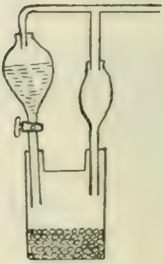
Drechsel attached to increase head of waste.



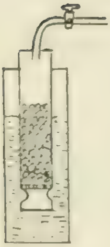
Kipp.



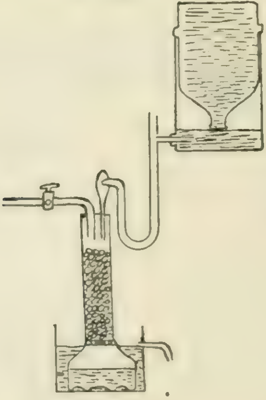
McCoy.



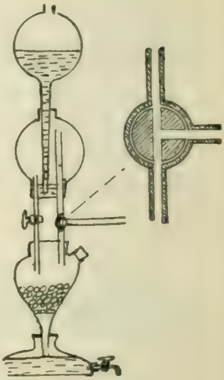
Kämmerer.



Heumann.



Parsons.



Teclu.

A GAS GENERATOR, IN FOUR FORMS,[a] FOR LABORATORY AND TECHNICAL USE.

By Norman Roberts,
Passed Assistant Surgeon, U. S. Public Health and Marine-Hospital Service.

A single German chemical-apparatus catalogue lists 23 different varieties of laboratory gas generator. Each one of these must obviously possess merit enough to make it more or less salable, none of them enough merit to displace the rest; and none of them are of anything like general applicability. Besides these there is an uncounted and daily increasing host of generators in the literature. Here is a wide-open invitation for some one to destroy this condition of pernicious equilibrium by combining, in one or a small number of forms of apparatus, the valuable features of the uncounted multitude of generators now in existence. New features, though not barred, are hardly necessary—there are so many serviceable old ones to be found among the rubbish.

The varieties of apparatus herein described are recognizable as hybrids from a number of well-known forms, which, among them, have most of the features recognized as being of value in a gas generator.

In the simplest form (fig. 1), that intended primarily for individual-student use, A represents a separating funnel containing dilute acid; B and C, a long straight large-caliber glass tube containing (in B) the solid material and (in C) gas or waste; M, a perforated cork paraffined in, separating B from C and supporting the solid material; D, a tall narrow museum jar containing water and spent acid; L, N, and P, perforated rubber stoppers; 1, 2, 3, and 4, cocks. The curved channels represent rubber and glass tubing.

The operation of this apparatus is as follows:

C and D having been filled with water through L or N, B with solid through N, and A with acid through P; all stoppers replaced and all cocks opened except 1, action is started by opening 1 carefully until acid drops steadily on the solid in B. The pressure of the generated gas, transmitted through the tube controled by cock 3 to the top of the acid in A, renders the rate of flow of the acid independent of the gas pressure. If the external resistance is only moderate, it is automatically balanced by the head of the liquid in D, the excess of liquid displaced by the gas wasting out automatically through cock 2. If the pressure of gas is greater than the head of the liquid in D, the gas will accumulate in C and bubble out through D and 2. This may be forestalled by closing 2 until liquid escapes from D no faster than acid enters through A. Escape of gas through D is then prevented by the liquid, and the gas pressure will rise until any reasonable external resistance is overcome, the rate of generation remaining unaffected. Too high pressure rarely occurs (since cock 2 should never be entirely closed), and is usually relieved by the blowing out of a stopper, or if a safety device is thought necessary, a U of mercury may be provided. A may be filled with acid by closing cock 3 and removing stopper P. If the external resistance is low and the stem of A is fairly long, the acid will continue to flow downward through the stem into B, and generation will be uninterrupted; but if the external resistance is high, it will be necessary to close cock 1 to prevent blowing back of gas during the filling with acid.

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[a] Manuscript submitted for publication, November 18, 1909.

This variety of the apparatus, while combining a number of valuable features in a cheap, compact, and easily improvised form, does not by any means fully develop the possibilities of this type of generator. By raising the acid container A, lengthening the tube *a* and providing it with a U at its lowest point, and eliminating the gas-carrying tube *c*, a very good apparatus of the "automatic" variety will result. This very closely resembles the Parsons generator, but considerable improvement can be made by substituting for the jar at D a bulbed U tube of considerable depth by introducing the acid into the middle of B instead of at the top, and by lengthening the container B upward, as shown in figure 2. By these modifications of the Parsons apparatus the possibility of flooding will be eliminated, the apparatus will work automatically against high external resistance, and the gas (being evolved at a point distant from the exit and being scrubbed on its way out by passage through a tower of active solid) will be comparatively free of acid and waste. For the proper working of this form of the apparatus, $tr < rs < tw$ (vertical distances; fig. 2); for the flow of acid must be stopped in time to prevent the gas from continuously wasting out through D; and if any such waste of gas occurs, it should take place through D, which is wide and will not be choked by a bubble of gas, rather than through *a*, where a bubble of gas might be difficult to dislodge, and where complete filling of the tube with gas would prevent automatic reestablishment of the flow. For an automatic generator capable of working against high resistance, the acid and waste tubes must of course be very long, and are best made of lead pipe.

It is possible to combine in a single apparatus the features of automatic and of "independent" generation of gas (the generation in one case being dependent on, and in the other independent of, the gas pressure within the apparatus), the apparatus being easily convertible from one type to the other, and thus having the advantages of both. In this form of the apparatus (fig. 3) a few extra parts are necessary or advantageous. X is an accessory acid container for recharging A with acid, without waste of gas, interruption of the flow, or admixture with air. Under low pressure, the acid runs from X into A by gravity; under high pressure it is necessary to force it in by means of air pressure produced by the hand bulb (or air pump) Y. 6 is a cock regulating the flow of acid between X and A. 7 is a sludge or drainage cock at the lowest point of the apparatus. H is a system of accessory drainage pipes and funnels to carry away the waste. 3 is a T-channel cock by which the apparatus is converted from "automatic" to "independent flow," and vice versa. With the cock in the first position (fig. 3, I) the gas pressure has access to the upper surface of the acid, and the flow is "independent." With the cock in the second position (fig. 3, II) the gas pressure acts only on the lower end of the acid column, at *v*, and the flow is automatic.

This form of the apparatus may be charged with solid (as well as acid) without interrupting or mixing air with the flow of gas, by the following procedure:

The apparatus having been working as "independent-flow," and A having become nearly empty of acid and full of gas, cocks 1 and 5 are closed, and fresh acid run in from X, the flow of gas being thus continued (in spite of cessation of generation) by the displacement of the gas in A. While this is going on, N is removed and the fresh solid (preferably somewhat moist from washing) is quickly introduced. N is replaced loosely and 1 opened slightly, enough gas being thus generated to force the air out past N. When the air is expelled, N is closed tightly, 5 opened wide at once, and 1 again adjusted to let in the acid at the desired rate.

In case automatic generation is not desired, the apparatus illustrated in figure 1 can be improved as illustrated in figure 4, which is the "combined" form with the automatic feature eliminated. Tube A is reduced to a short nearly horizontal length with no U, the complicated cock 3 is eliminated and solid-container B is shortened. The lower U of the apparatus (C, D′, and D) is replaced by removable small caliber rubber tubing, D and D′ consisting of a spherical funnel. This U should be quite deep to prevent with reasonable certainty the blowing back of the gas without the use of the chamber C. As it carries dead waste and not live acid, it will probably give little trouble from leakage. Cock 2 should be located as indicated in figure 4, whether the rubber U tube is attached or not. When the apparatus is working without the rubber U, cock 2 must be adjusted so that the waste escapes neither faster nor slower than the acid enters.

In any of the forms of this apparatus the back pressure of the waste may be increased by attaching small caliber tubing (or a series of Drechsel bottles connected backward) to *b* so as to raise *s*. In the automatic form (fig. 2) the fresh-acid pressure may be increased by inserting into the mouth of A a perforated rubber stopper carrying a long stemmed separatory funnel reaching to the bottom of A and supplying acid. These makeshifts are for the purpose of increasing the range of the automatic actions of the apparatus beyond the ordinary on special occasions; but they are apt to upset the equilibrium of the apparatus and have to be watched.

Relative advantages of the different forms.—The first form is the best for small work. It is simple, compact, cheap, and easily improvised, having easily regulated flow of acid and gas, automatic discharge of waste at low pressures, economy of materials, ease of charging and cleaning (largely self-cleaning), and ability to stand indefinitely without waste of material, clogging, or deterioration. It has also the feature, unusual in a small apparatus, of generating against any reasonable external resistance, so that the gas may be as thoroughly purified as desired by passing through a number of absorption flasks without affecting the rate of evolution of the gas.

The second form (fig. 2) is an improved Parsons automatic generator, incapable of flooding, giving a clean gas, and by proper proportioning capable of working automatically, as to flow of gas and waste, under a wide range of pressures.

The third form (fig. 3), while the most complicated and expensive (though much less so than many other generators having fewer features of merit), has all the advantages of both automatic and "independent" working, together with the possibility of indefinitely prolonged evolution of unmixed gas.

The fourth form (fig. 4) is an improved first form, having all the advantages of the third form except automatic generation, together with a simpler construction.

A number of recent gas generators provide for the automatic discharge of the waste through a mercury trap. This device is not so good as a U tube filled with the waste, since it requires careful adjustment, is apt to get out of order, and does not provide any elasticity in the matter of gas capacity.

There are appended hereto diagrams representing a number of generators which may be regarded as "ancestral forms" of which our generator is a hybrid. As will be seen, the Kämmerer generator has the feature of constant acid flow irrespective of gas pressure; the Parsons takes in the fresh acid and discharges the waste through curve-down siphons or their equivalent, and it also uses a tall column of solid instead of a short column; the Kämmerer, Parsons, and McCoy types apply the fresh acid in a small stream, as distinguished from a large mass of partly used acid, at a point considerably above the bottom of the active solid; in the Heumann form the spent acid is blown downward away from the active solid when the stream of escaping gas is shut off, and the gas which accumulates is a reserve which is made use of the next time the flow is turned on before the generation of fresh gas is established, as is done in the Kipp form also; the Teclu apparatus has a reservoir of gas which can be used to maintain the flow while the apparatus is being recharged with acid or solid. It will thus be seen that our apparatus borrows one or more valuable features from each of these generators. The only apparently new features are the tall column of solid extending upward from the point of entrance of the acid to a level above that of the acid in the reservoir (thus insuring against flooding and scouring the gas), and the simple device for combining in a single apparatus the advantages of automatic and independent flow generators.

A modification of this apparatus may be used successfully for the generation of gases by the interaction of two fluids, e. g., HCl from HCl. Aq. and H_2SO_4, the tower being filled with an inactive porous material such as pumis, and the two fluids being fed upon this porous material from two separate jets, such as the mechanism described for the regular form.

In the case of the generation of HCl above mentioned, the sulfuric acid could with advantage be fed in at the top of the column and the HCl. Aq. introduced at a point lower down.

The following are possible applications of this generator in some of its forms:

Hydrogen, from zinc and acid, or from aluminum and alkali.

Hydrogen sulfid, from ferrous sulfid and acid.

Carbon dioxid, from insoluble carbonates and acid.

Sulfur dioxid, from sulfites and acid.

Acetylene, from calcium carbid and acid.

Methane, from aluminum carbid and acid, (warm).

Ammonia, from alkali and ammonium chloride solution (with waste acid sifon shut off).

Oxygen, from alkali peroxids and acid or water.

Hydrochloric acid, from its solution and concentrated sulfuric acid.

Carbon dioxide, from solutions of carbonates, and acids.

Formaldehyd, from its concentrated solution and potassium permanganate. (?)

For use in many laboratories small iron cylinders of compressed gas are much to be preferred to any form of generator. Pure compressed H_2, O_2, CO_2, SO_2, N_2O, Cl_2, and perhaps other gases are more or less easily obtained; but for no very evident reason, compressed H_2S is rarely or never used or catalogued, although this gas is one of the most important chemical reagents and its small-scale generation and purification is one of the greatest nuisances that the laboratory worker has to content with. According to Moissan[a] hydrogen sulfide is easily liquified and the gas above the liquid has a pressure of 14.6 atmospheres at 11.1°C. The liquid is colorless, very mobile, specific gravity 0.86, and the heat of vaporization 4.23 cal. Its freezing point is —86.°C.

I am indebted to Dr. Atherton Seidell, to Mr. Elias Elvove, and especially to Mr. F. A. McDermott, all of the Hygienic Laboratory, for advice and assistance.

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[a] Traite de Chimie Minerale, Paris, 1905, Vol. I, p. 342.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi.* By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth decennial revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of etheral salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

*No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

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*No. 41.—Milk and its relation to the public health. By various authors.

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No. 45.—Further studies upon anaphylaxis. By M. J. Rosenau and John F. Anderson.

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No. 50.—Further studies upon the phenomenon of anaphylaxis. By M. J. Rosenau and John F. Anderson.

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No. 60.—A study of the anatomy of Watsonius (n. g.) Watsoni of man, and of 19 allied species of mammalian trematode worms of the superfamily Paramphistomoidea. By Ch. Wardell Stiles and Joseph Goldberger.

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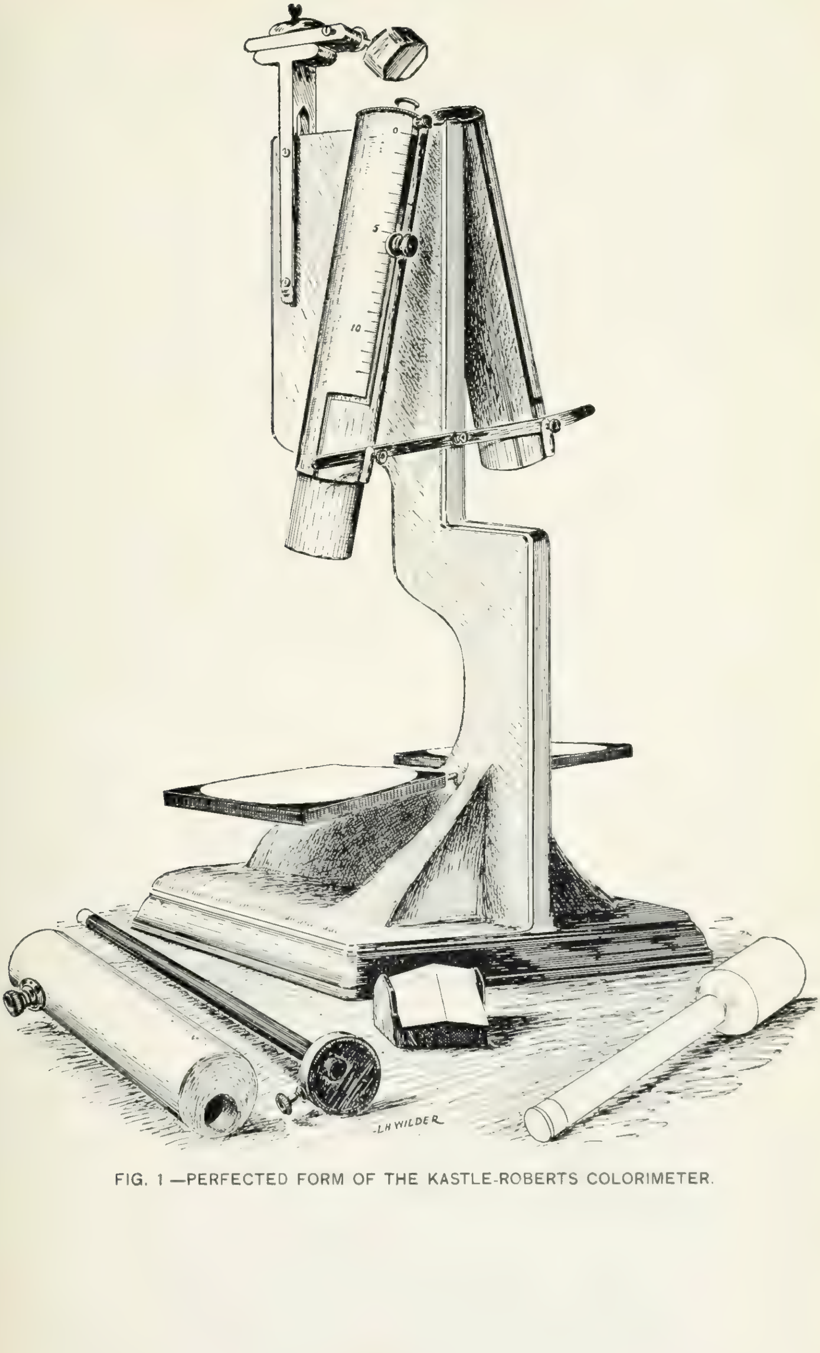

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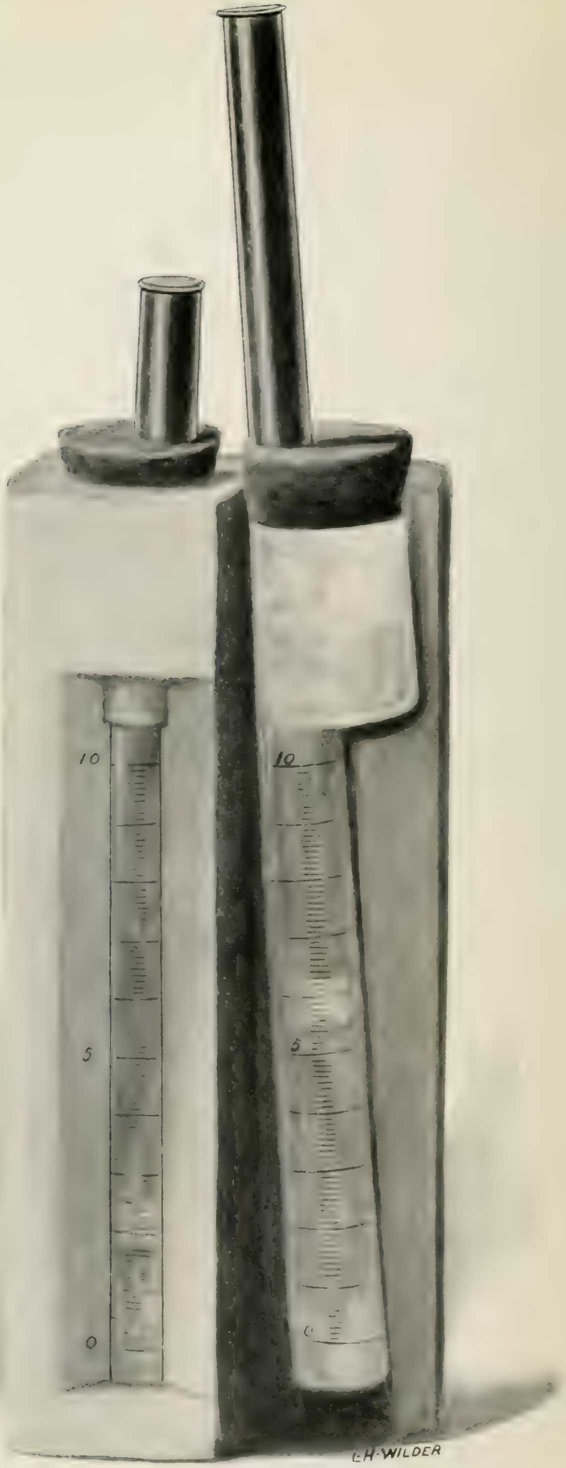

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HYGIENIC LABORATORY.—BULLETIN No. 67

JUNE, 1910

THE SOLUBILITIES OF THE PHARMACOPŒIAL ORGANIC ACIDS AND THEIR SALTS

BY

ATHERTON SEIDELL

WASHINGTON
GOVERNMENT PRINTING OFFICE
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THE SOLUBILITIES OF THE PHARMACOPŒIAL ORGANIC ACIDS AND THEIR SALTS.[a]

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INTRODUCTION.

Among the physical properties of the chemical compounds of the Pharmacopœia none have apparently received more attention at the hands of the compilers of the book than the solubilities. The reasons for this are no doubt due to the fact that such data are known to be of practical value to the pharmacist on the one hand and to the physician on the other. The former uses the figures as a guide in compounding every mixture he is called upon to make; the latter must take this property into consideration in the selection of many substances he prescribes for particular purposes. It is to the chemist, however, who is concerned with the preparation, purification, and accurate determination of chemical compounds, that solubility data are of greatest importance. Most analytical methods are more or less directly dependent upon some solubility relation; furthermore, the technical production of a large proportion of the chemicals and drugs now in use is made possible only by means of known solubility characteristics.

The very fact of the diversified uses which solubility data fulfill accounts for the great variation in the accuracy of the results reported in the literature. One investigator may have desired a solvent for a new compound, while another may have been concerned with the solubility of a precipitate obtained in making a molecular weight determination. Probably very few have worked under identical conditions, either of purity of the substance or of the solvent, of equally exact temperature regulation or care in securing the saturation of the solution. It is easy, then, to understand why equally creditable reference books may differ so widely in the values, gathered from the literature, for the solubility of the same compound.

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[a] Manuscript submitted for publication May 18, 1910.

Of reference books for this class of data, the pharmacopœias of the various countries are particularly subject to differences in the values quoted. This fact was very forcibly demonstrated by a compilation made by me [a] some two years ago of the solubilities and other physical constants reported by the latest pharmacopœias and pharmaceutical reference books. The extent of the variations for some of the compounds, especially the inorganic salts, was not very great; for many others, however, there was no way of deciding which of the reported values were nearest the truth. This was especially the case with the alkaloids, and to a large extent with the other organic compounds of the pharmacopœias. The matter was still further complicated by the fact that very concordant results were sometimes reported, although the standards of temperature, purity, etc., indicated that such good agreement was not justified, thus showing that the compilers of these works of reference frequently borrow from one another without giving the information which would enable the reader to consult the original source of the data. These difficulties and many others made it impossible to gather from the compiled solubility values the particular ones which might be considered the most reliable; in fact, the great number of different figures only added to the uncertainty and made it apparent that the publication of the compilation could serve no other purpose than to call attention to the need for renewed investigations of the solubilities of the pharmacopœial compounds. The original intention of publishing this compilation as a bulletin from this laboratory was therefore abandoned in favor of publishing only such values as we might be able to determine with accuracy or select from the chemical literature as of undoubted reliability.

Although, as has been pointed out above, the solubility data for alkaloids and their salts are perhaps most in need of careful revision, the lack of a sufficient amount of material and of satisfactory methods for their accurate determination made it advisable to leave this class of substances for a later study and take up first a group which offered less difficulties and for which, after the alkaloids, there appeared most need for careful investigation. This group is that of the organic acids and their salts, and is composed of about 40 compounds, the solubilities of very few of which, in the solvents prescribed by the Pharmacopœia, have been reported in recent chemical literature. For this group of compounds, therefore, original determinations were required for practically every member.

In the present bulletin the arrangement is alphabetical according to the names of the acids, with the salts of each immediately following. The solubility of each acid has been determined in water and aqueous alcohol solutions of varying concentrations and in a large number of the common organic solvents. For the salts, determinations only in the water and aqueous alcohol solutions are given. Although the strength of the alcohol used as the solvent when the solubility in this substance is mentioned in the Pharmacopœia is generally understood to be that of the official strength, viz. 92.3 per cent by weight, it is evident that from a practical standpoint the solubility of substances in alcohol of slightly and also widely different strengths would be useful in many cases. Furthermore, the determination of the solubility in alcohols of different strengths does not require a very much greater amount of work than the determination in only one strength, since in the former case the result in each solvent is as satisfactory a check upon the results found for the other strengths of alcohol, when all are plotted on cross-section paper, as would be an equal number of duplicate determinations in alcoholic solutions of the same concentration. The results for the whole series of alcoholic concentrations give curves which, by their direction, whether horizontal or greatly inclined, show the extent of the influence of small differences in alcoholic concentration upon the solubility at any given point. The added value of the results over the determination in alcohol of official strength only, seems therefore, sufficient to warrant the expenditure of the additional time required. There is still another point worthy of consideration, and that is the possibility of learning more in regard to the nature of solution in mixed solvents—whether it is a mechanical relation proportional to the amounts of the two liquids present or controlled by some other factor not as yet known.

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[a] For a more complete description of this compilation, with comments, see Proc. Am. Pharm. Assoc., **55**, 473–479, 1907.

The results are given in the tables in all cases in the terms in which solubility data are usually expressed in the chemical literature, and in addition the equivalent values are expressed as customarily found in the pharmaceutical literature, viz, the amount of solvent required to dissolve a unit amount of the dissolved substance. Although this latter mode of expressing solubility results has been used for many years by pharmacists, it is unscientific and not even as useful for practical purposes as the percentage basis adopted by chemists. As an illustration, pharmacists are usually called upon to compound their mixtures in certain definite amounts, and therefore the quantity of the dissolved substance which will be contained by the given volume of the solution is certainly of more practical interest to them than the knowledge of the amount of the solvent required for one part of the given substance. It would therefore appear of considerable advantage to pharmacists to abandon their antiquated mode of expressing solubility results and adopt the more rational percentage or unit of solvent basis in their pharmacopœias and pharmaceutical reference books.

EXPERIMENTAL METHODS.

In all cases attempts were made to purify the material used for the solubility determinations at least to the extent required by the purity rubric of the Pharmacopœia. In a very few cases this could not be done with certainty, but in the others was accomplished successfully. The samples were analyzed quantitatively and the results reported in connection with the solubility determinations in most cases. It is believed that in those cases where the presence of certain very small amounts of unknown impurities were indicated by the analyses they were not sufficient to exert an appreciable effect upon the solubility values reported. This is concluded from the observation that in several instances determinations made upon samples containing considerable more impurity than shown by the analyses of the majority of the following compounds did not differ appreciably from the results obtained with the material of the highest purity.

All of the solubility determinations, except those for the liquid compounds, ethyl acetate, methyl salicylate, and oleic acid, were made under strictly identical conditions. A constant temperature water bath, regulated to within + 0.05° of 25° C., was used. The saturated solutions were prepared by mixing the solvent and the salt in thick-walled test tubes of about 15 to 20 cubic centimeters capacity, closed with well-washed rubber stoppers. Glass-stoppered cylinders were used for the determinations made in organic solvents. The test tubes or cylinders, as the case might be, were attached to a rotating frame immersed in the water bath and revolved upon the axis parallel to the diameter of the test tube at a rate of about six revolutions per minute. Equilibrium was assured in most cases by analyzing solutions which had been agitated for different lengths of time. In those cases where only one series of determinations was made the time of shaking was continued long enough to insure complete saturation. Care was taken that a sufficient excess of salt was in contact with every solution at the saturation point.

The time allowed in the present experiments for obtaining saturated solutions is not necessarily an indication of the time that is actually necessary. It is very probable that the saturation point could be reached in very much shorter time than indicated in most of the following experiments, but since the object was to ascertain the true solubility in the several cases care was taken that sufficient time for obtaining the maximum solubility should be allowed in every case.

A sufficient quantity of each of the aqueous alcoholic solutions for all of the experiments was prepared at the beginning and the specific gravities of these solutions carefully determined by the pycnometer method. These determinations were made upon the solutions cooled to 15°, and the weight per cents of absolute alcohol (C_2H_5OH) corresponding to each was read from the U. S. Pharmacopœia alcohol table.

The general plan of analyzing the saturated solutions was as follows: At the end of the period of rotation the tubes were placed in an upright position in the bath until the undissolved salt had settled to the bottom. Each tube was then removed to the laboratory table in a beaker filled with the water of the constant temperature bath, and immediately thereafter the supernatant clear liquid was siphoned directly into a 10-cubic centimeter pycnometer with the aid of a suction pump. The suction siphon used for this purpose consisted of a closed glass cylinder just large enough to contain the pycnometer. The siphon tube passed through the stopper of this cylinder directly into the mouth of the pycnometer. Another glass tube through the stopper served to connect the apparatus with the suction pump. In those cases where there was suspended matter remaining in the saturated solution a small tube containing a plug of cotton or of glass wool in the case of organic solvents was attached to the end of the siphon which was introduced into the saturated solution. After filling, the pycnometer was weighed and, in the case of salts which could be dried without decomposition, the saturated solution was transferred to a weighing bottle, evaporated to dryness, and the residue weighed. With the organic acids which could be titrated, or with the salts which decomposed on drying, the saturated solutions were transferred to graduated flasks and aliquot portions analyzed in the most convenient manner.

The results in all cases were calculated to the grams of salt dissolved in 100 grams of the saturated solution. The figures were plotted as the abscissæ and the weight per cents of alcohol in the several solvents as the ordinates on cross-section paper. The curve drawn through the several points represents the solubility of the particular salt in the aqueous alcoholic solutions of increasing content of alcohol. From this curve the figures corresponding to regular intervals of alcoholic strength of the solvent were read.

The specific gravities are in all cases the weight of a given volume of the saturated solution or of the alcoholic solvent divided by the weight of an equal volume of water at the same temperature, i. e., $d\frac{25}{25}$, or for the alcoholic solvents, $d\frac{15}{15}$.

ACETIC ACID AND THE ACETATES.

The solubility of acetic acid.—Although this acid mixes with water, alcohol, and the ordinary organic solvents in all proportions, numerous experiments have shown that when a given quantity is added to a mixture of equal volumes of water and an immiscible solvent there is a distribution between the two layers which varies considerably with the nature of the immiscible solvent. To illustrate the differences more clearly, the following table (No. I) has been compiled from the available results. In this table the organic solvents have been arranged in the descending order of their power of withdrawing acetic acid from its aqueous solution. It will be seen that amyl alcohol exerts by far the strongest solvent action, chloroform comes next, with less than one-sixth the strength, and then follow bromoform, benzene, toluene, the xylenes, carbon tetrachloride, and carbon bisulphide in a gradually descending scale of efficiency as solvents for acetic acid. A comparison of these results with those for the other pharmacopœial acids in some of the above solvents show that the general order of their solvent action is nearly the same for the whole series of acids.

TABLE No. I.—*Distribution of acetic acid between water and several immiscible organic solvents at 25°.*

[Compiled from the results of Herz and Fischer, Ber. **37**, 4747, 1904; **38**, 1140, 1905; Herz and Lewey, Z. electro. Chem., **11**, 818, 1905, and Rothmund and Wilsmore, Z. physik. Chem., **40**, 623, 1902.]

CH_3COOH per 100 c. c. H_2O layer.	CH_3COOH per 100 c. c. of the immiscible layer.								
	Amyl alcohol.	$CHCl_3$.	$CHBr_3$.	C_6H_6.	$C_6H_5CH_3$.	$C_6H_4(CH_3)_2$ (o) or (p).	$C_6H_4(CH_3)_2$ (m).	CCl_4.	CS_2.
Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
2	1.847	0.089	....	....	....	....	....	....	....
5	4.587	0.450	....	0.13	0.12	0.24	0.06	....	....
10	[a] 9.100	1.430	....	0.42	0.33	0.48	0.30	....	....
20	....	5.100	1.5	1.55	1.13	1.13	0.95	....	....
30	....	10.200	3.0	3.03	2.26	2.15	1.91	1.8	....
40	....	15.300	4.8	4.95	3.73	3.40	3.04	3.0	....
50	....	21.900	7.8	....	5.84	5.10	4.65	4.8	....
60	....	....	12.0	....	8.34	7.27	6.65	5.8	2.3
70	....	....	27.0	....	....	12.52	....	12.0	3.0
80	....	....	....	....	....	....	....	....	5.4

[a] Estimated.

The solubility of ethyl acetate in aqueous alcohol solutions.—The U. S. Pharmacopœia description of this product specifies that it consist of about 90 per cent by weight of ethyl acetate and 10 per cent of alcohol containing a little water. Since, however, the alcohol which a given sample of the compound may contain is wholly soluble in water, it follows that the saturation of the solution is due only to the ethyl acetate present. For the purpose of the determination of the solubility of the pharmacopœial acetate it is therefore evident that the object will be more satisfactorily attained by the investigation of the solubility of the pure compound in aqueous alcohol solutions of increasing concentration. Studies of this character were made some years ago by Bancroft [a] in the manner that ethyl acetate was gradually added to mixtures of known volumes of alcohol and water until an opalescence was observed. The experiments were made at 20°. Furthermore, the water and ethyl acetate used were each previously saturated with the other. It would therefore be necessary to apply a calculated correction factor to the results in order to bring them to the terms desired for the present purposes. In view of the possible uncertainty which might arise through such calculations it appeared more desirable to make new determinations upon materials of highest purity.

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[a] Phys. Review, **3**, 122, 131, 1895–96.

The ethyl acetate used was prepared from the Kahlbaum product by allowing it to stand over calcium chloride for a day, filtering, and distilling. That part of the liquid, amounting to nearly one-half of the whole, which distilled between 75° and 76° (corrected) was reserved for the solubility determinations. The specific gravity of this fraction was 0.8984 at 15°, 0.8948 at 20°, and 0.8915 at 25°. The aqueous alcohol solutions were those prepared, as already mentioned (p. 10), for the solubility determinations of all the compounds described in this bulletin. The method used in the present case was as follows: The accurately measured portions of the aqueous alcohol solutions were placed in small Erlenmeyer flasks and brought to as near 25° as possible; a burette was filled with the purified ethyl acetate, which had been allowed to remain in the constant temperature bath at 25° for an hour or longer. The temperature of the room at the time the determinations were made was 22° to 24°. The ethyl acetate was added very gradually to the aqueous alcohol and the solution shaken between each addition. The saturation point was indicated by the appearance of a distinct opalescence, less than 0.05 cubic centimeter being sufficient to cause the change from clear to cloudy condition in most cases. Satisfactory concordance in duplicate determinations was obtained. The flasks containing the slightly opalescent saturated solutions were suspended in the constant temperature bath for an hour or longer, after which time the specific gravities of the solutions were determined by the pycnometer method. The results of the determinations are given in Table No. II and show that ethyl acetate is completely miscible with aqueous alcohol solutions of about 40 weight per cent or more concentration. The curve for this compound in Plate No. I shows that the solubility increases gradually at first and then very rapidly, with increase of alcoholic strength of the solvent.

TABLE NO. II.—*The solubility of ethyl acetate in aqueous alcohol solutions at 25° C.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$CH_3COOC_2H_5$ per 100 c. c. solvent.	$CH_3COOC_2H_5$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.			
			c. c.	*Grams.*
Dist. H_2O	0.0	0.9993	9.6	7.90
0.9856	8.9	0.9897	11.8	9.64
0.9752	17.0	0.9690	17.0	13.45
0.9628	26.4	0.9414	49.0	31.19
0.9545	32.0	0.9268	86.1	44.58
0.9359	41.7	....	∞	∞

CALCULATED RESULTS.

The above results plotted on cross-section paper gave a curve from which the following figures were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$CH_3COOC_2H_5$ per 100 c. c. solvent.	$CH_3COOC_2H_5$ per 100 grams.		Solvent to dissolve 1 gram $CH_3COOC_2H_5$.
			Saturated solution.	Solvent.	
		c. c.	*Grams.*	*Grams.*	*Grams.*
0	0.999	10.0	7.9	8.6	11.10
5	0.993	10.5	8.7	9.5	10.50
10	0.986	12.0	9.8	10.9	9.20
15	0.974	15.0	11.7	13.3	7.50
20	0.960	27.0	16.4	19.6	5.10
25	0.945	44.0	27.0	37.0	2.70
30	0.931	70.0	40.0	66.7	1.50
35	0.918	125.0	57.0	132.5	0.75
40	....	∞	∞	∞	....

Since the effect of the alcohol upon the solubility of the acetate is gradual at first, it follows that the solubility of the pharmacopœial product containing approximately 10 per cent alcohol is not very different from the value shown above for the pure compound. Thus if pharmacopœial ethyl acetate containing 10 per cent alcohol be added to 100 grams of pure water the necessary amount to form a saturated solution would carry with it only enough alcohol to make an approximately 1 per cent aqueous alcoholic solvent; therefore the amount of the pharmacopœial acetate required would be only about one-tenth more than the quantity of the pure compound indicated in the above table—i. e., 8.6 + 0.86 grams = 9.66 per 100 grams water or 10.3 weight parts of water per 1 part of pharmacopœial ethyl acetate. This figure is somewhat higher than that quoted by the present Pharmacopœia, viz, 9.0 parts water per 1 part acetate.

The solubility of ethyl acetate in water at different temperatures.—Two determinations of the solubility of ethyl acetate in water at 20° and 28° are reported in the literature respectively by Bancroft[a] and

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[a] Loc. cit.

Euler.[a] The results in terms of grams of ethyl acetate per 100 cubic centimeters of water are, at 20°, 8.25 grams, and at 28°, 7.26 grams. Since these figures indicate that this compound shows the rather unusual property of a decreasing solubility with increase of temperature, and also since my value for 25° did not fall between these two for a higher and lower temperature, it appeared desirable to investigate the solubility of this compound in water over a range of temperature.

The determinations were made by titrating to appearance of opalescence 50 cubic centimeter portions of distilled water, of approximately the desired temperature, with the purified ethyl acetate described above and noting the exact temperature of the mixture immediately after the saturation point was reached. The actual readings were made between 10° and 40°, but the regularity of the curve permitted the extention of the values beyond this range as shown in the accompanying table, No. III.

TABLE NO. III.—*The solubility of ethyl acetate in water at temperatures between 0° and 55° C.*

Temperature.	$CH_3COOC_2H_5$ per 100 c. c. H_2O.	$CH_3COOC_2H_5$ per 100 grams H_2O.	$CH_3COOC_2H_5$ per 100 grams saturated solution.
° C.	*c. c.*	*Grams.*	*Grams.*
0	13.20	11.8	10.53
5	12.10	10.8	9.74
10	11.30	10.1	9.15
15	10.70	9.5	8.71
20	10.10	9.0	8.27
25	9.60	8.6	7.90
30	9.20	8.2	7.61
35	8.90	7.9	7.39
40	8.60	7.7	7.17
45	8.35	7.4	7.00
50	8.10	7.2	6.81
55	7.94	7.1	6.70

In addition to the above determinations of the solubility of ethyl acetate in water and alcohol solutions, one determination was made of the solubility of water in ethyl acetate giving the following results:

Cubic centimeters of H_2O per 100 cubic centimeters $CH_3COOC_2H_5$ at 25°=4.8.
Grams of H_2O per 100 grams $CH_3COOC_2H_5$ at 25°=5.38.
Specific gravity of saturated solution at 25°=0.9059.

The solubility of lead acetate in aqueous alcohol solutions.—Since no quantitative method is prescribed by the pharmacopœia for the determination of the purity of lead acetate, the following plan, based upon the insolubility of lead sulphate, was used. A weighed amount of 0.5 to 1.0 gram of the sample is dissolved in about 50 cubic centimeters of distilled water and dilute H_2SO_4 added to the cloudy solution until no further precipitation occurs. The flocculent white precipitate is filtered on a weighed Gooch crucible, washed with a little water, and finally with a few cubic centimeters of alcohol, dried in an oven at about 150°, and weighed as $PbSO_4$. The solubility of lead sulphate in water is stated to be 0.041 gram per liter, and therefore in case the filtrate and washings in the determination as above outlined amount to 100 cubic centimeters the error, when a 0.5 gram sample is employed, may amount to 1 per cent. It is probable, however, that the excess of sulphuric acid used for the precipitation would lower this error somewhat.

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[a] Z. physik. Chem., **31**, 360, 1899.

A number of commercial samples of lead acetate examined in this laboratory by the above method gave results showing, when calculated to $Pb(C_2H_3O_2)_2 + 3H_2O$, a variation between 101.6 and 106.1 per cent. The pharmacopœial requirement of 99.5 per cent $Pb(C_2H_3O_2)_2 + 3H_2O$ appears, therefore, to be practically unattainable. None of the samples gave a clear solution with recently boiled distilled water, as required by the pharmacopœia. Each of them smelled strongly of acetic acid, and as far as could be judged differed only in the extent to which the volatilization of this constituent had proceeded. It would be interesting to determine the vapor pressure conditions under which a compound of the theoretical composition exists, but for the purpose of the present solubility determinations such a study does not appear of particular importance.

The sample of lead acetate used for the following experiments was the one which contained upon analysis the amount of lead (57.9 per cent) corresponding to 106.1 per cent $Pb(C_2H_3O_2)_2 + 3H_2O$. In appearance it differed from the one containing 101.6 per cent of the salt only in being less moist, and would probably be selected by most persons as the better sample. Although it might be possible to prepare a quantity of lead acetate of the theoretical composition, calculated from the determination of the lead, it is doubtful whether such a sample would really be of 100 per cent purity, since adhering moisture and acetic acid would probably be present, and the true composition of the salt in hand might vary as far in the opposite direction as the present sample goes toward the basic salt. The question would then resolve itself into what effect the difference in amounts of acetic acid might have upon the solubility in the aqueous alcohol solutions and would require solubility determinations in solutions containing varying amounts of free acetic acid. The following results show the position of the curve for a sample of lead acetate containing a certain amount of the basic salt. Another sample would no doubt give slightly different results, but, as pointed out above, the same uncertainty would be present in both cases.

Two series of determinations were made in the present case. The second was for the particular purpose of establishing the point of change of solid phase from the hydrated to the anhydrous salt. The lengths of time of the shaking for reaching the saturation point were respectively two and four days. The saturated solutions were perfectly clear in all cases, but upon dilution, preparatory to making

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the lead sulphate determination, each became cloudy. The solid phase in the solutions of lower alcoholic content consisted of clear crystals, and in the solutions of 81.5 per cent or more alcohol it was of a very fine, silky, fibrous appearance. The line of demarcation between the two forms was very sharp. An analysis made of the undissolved residue remaining in the tube of 81.5 per cent by weight alcohol showed it to consist of the anhydrous salt, thus confirming the conclusion based upon the difference in appearance of the solid phase in the tubes of the higher alcoholic concentrations.

The saturated solutions were analyzed by precipitation with dilute H_2SO_4 and weighing the lead sulphate, as already described. The results are shown in Table No. IV and the solubility curve in figure 1.

TABLE NO. IV.—*Solubility of lead acetate in aqueous alcohol solutions at 25° C.*

EXPERIMENTAL RESULTS.

Solvent.		Specific gravity of saturated solution at 25°.	Salt per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.343	42.61
0.9856	8.9	1.285	38.41
0.9545	32.0	1.150	28.45 [a]
0.9164	51.0	1.052	21.49
0.8718	70.2	0.952	14.42
0.8441	81.5	0.902	9.42
0.8234	89.6	0.827	1.80
0.8190	91.4	0.821	1.27 [b]
0.8048	96.2	0.802	0.46
0.7941	99.9	0.791	0.40

[a] Figures show grams of $(CH_3COO)_2$ $Pb+3H_2O$.
[b] Figures show grams of $(CH_3COO)_2$ Pb.

CALCULATED RESULTS.

The above results yielded a curve from which the following figures were read or calculated:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	Salt per 100 grams.		Solvent to dissolve 1 gram salt.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.343	42.6	72.5	1.38
10.0	1.275	37.7	60.5	1.65
20.0	1.215	33.3	50.0	2.00
30.0	1.157	29.2	41.3	2.42
40.0	1.105	25.5 [a]	34.2 [a]	2.92
50.0	1.055	21.8	27.9	3.59
60.0	1.002	18.2	22.3	4.49
70.0	0.955	14.5	17.0	5.90
80.0	0.907	11.0	12.4	8.09
81.0	0.905	10.5	11.7	8.52
85.0	0.855	4.0	4.2	24.00
90.0	0.826	1.6 [b]	1.6 [b]	61.50
[c] 92.3	0.817	1.1	1.1	89.90
95.0	0.806	0.6	0.6	131.60
100.0	0.790	0.4	0.4	249.00

[a] Figures show grams of $(CH_3COO)_2Pb+3H_2O$.
[b] Figures show grams of $(CH_3COO)_2Pb$.
[c] U. S. Pharmacopœia strength.

The solubility of potassium acetate in aqueous alcohol solutions.—The pharmacopœial method of analysis of the sodium and potassium salts of organic acids consists in incinerating the sample, extracting the charred residue, with water, and titrating the filtered solution with 0.5 N HCl, using methyl orange as the indicator. This procedure, although perhaps satisfactory for some of the salts to which it is applied, requires a modification for the attainment of accurate results in the case of certain of the others, notably sodium benzoate. A number of experiments with this salt (for details see p. 28) showed that a part of the alkali can not be successfully extracted from the charred residue, and therefore low results are obtained unless the unburned carbon is subsequently ignited and the residue dissolved in water and mixed with the main extract of the ignited sample. Although no experiments have been made with other salts than sodium benzoate to ascertain the extent of the error due to the neglect of this modification, qualitative observations indicate that it may arise with practically all of these salts. This procedure was therefore followed in all the analyses of these compounds reported in the following pages.

Two samples of potassium acetate from different sources contained, respectively, 96.4 and 96.7 per cent CH_3COOK. Since this compound absorbs moisture very rapidly, it appears that this low result might be due to adhering water. That this was not the case, however, was shown by analyzing one of the samples after drying in an air bath at 160° for six hours and overnight in a vacuum desiccator containing concentrated H_2SO_4. The second analysis showed a purity of only 97.6 per cent.

In order to obtain a product of greater purity than the purchased samples, a portion of one of them was recrystallized from hot 95 per cent alcohol, washed with a little alcohol, and dried in a hot air bath at 135° to 140° for fifteen hours. Duplicate determinations showed the material to contain 99 per cent CH_3COOK. The free acid present corresponded to less than 0.2 per cent CH_3COOH. The recrystallized sample was used for the solubility determinations contained in the accompanying table, No. V.

Two series of determinations were made. In the one the period of shaking was two and in the other four days. Some difficulty was experienced in withdrawing sufficient amounts of the clear saturated solution for analysis, since the undissolved excess of salt settled imperfectly. The weighed saturated solutions were transferred to weighing bottles, evaporated to dryness in an air bath, and dried to nearly constant weight at a temperature of 120° to 140°. Portions of each of the residues were analyzed, as above described, for the original samples and the amount of anhydrous CH_3COOK present in each calculated. The proportion of the anhydrous salt in the several residues varied from 94.3 per cent in the case of solution in water alone to 99.1 per cent in the absolute alcohol solution.

Table No. V.—*The solubility of potassium acetate in aqueous solutions of ethyl alcohol at 25° C.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	CH_3COOK per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.417	68.73
0.9545	32.0	1.336	67.74
0.9164	51.0	1.255	62.50
0.8718	70.2	1.154	55.30
0.8234	89.6	0.997	35.70
0.8048	96.2	0.909	22.90
0.7941	99.9	0.857	14.50

CALCULATED RESULTS.

The above determinations plotted on cross-section paper gave a curve from which the following figures were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	CH_3COOK per 100 grams.		Solvent to dissolve 1 gram CH_3COOK.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.417	68.7	219.6	0.455
20.0	1.363	68.7	219.6	0.455
40.0	1.302	65.8	192.4	0.520
50.0	1.260	63.2	171.8	0.582
60.0	1.210	59.6	147.5	0.678
70.0	1.156	54.2	118.3	0.855
80.0	1.085	46.7	87.6	1.140
90.0	0.990	34.6	52.9	1.890
92.3	0.957	30.5	43.9	2.280
95.0	0.922	25.5	34.2	2.920
100.0	0.850	14.0	16.3	6.140

The solubility of sodium acetate in aqueous alcohol solutions.—The analyses of the salt and of the residues obtained from the saturated solutions were made by the pharmacopœial method, modified as described under potassium acetate. It should be mentioned, however, that with this salt all of the carbon in the residue could be burned, if enough heat were applied, and the white residue dissolved in water and titrated directly. The sample used for the solubility determinations was in the form of moderately sized clear crystals and contained 99.0 per cent $CH_3COONa+3H_2O$. The impurity was evidently adhering moisture, since the analyses of the dried residues from the saturated solutions showed them to contain 99.5 or more per cent CH_3COONa. The sample was neutral to litmus and practically so toward phenolphthaleine, since about 3.0 grams dissolved in 20 cubic centimeters water required only 3 drops of 0.1 N NaOH to give the strong pink color of phenolphthaleine.

Two series of determinations were made, the time of shaking being respectively two and four days. No change in the solid phase was apparent in any of the tubes. The weighed saturated solutions were transferred to weighing bottles, evaporated to dryness in an air bath at about 120°, and dried for three to five hours at 150°. Portions of the resulting residues of anhydrous sodium acetate were analyzed and shown to contain approximately 99.5 per cent CH_3COONa. The weights of the anhydrous salt were calculated to the hydrated compound, since it was evident that the salt had dissolved as such without change. The results are given in Table No. VI and the curve in figure 1.

The solubility of sodium acetate in water and in alcohol solutions of several concentrations has been determined by G. Schiavon (Gazz. chim. ital. (Roma), 1902, *32* II, p. 532), but the solutions were agitated only at intervals and the temperature maintained constant for comparatively short periods of time. The results are somewhat higher than those here shown, due probably to the fact that the saturation point was approached from above and equilibrium had not been reached. One determination in water at 31.5°, reported by Köhler (Z. Ver. Zuckerind, 1897, *44*, 447), is evidently much too low as compared with the results of Schiavon and the present value for 25°.

TABLE NO. VI.—*The solubility of sodium acetate in aqueous solutions of ethyl alcohol at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$CH_3COONa + 3H_2O$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.209	55.75
0.9856	8.9	1.162	53.22
0.9545	32.0	1.104	45.59
0.9164	51.0	1.034	36.44
0.8718	70.2	0.941	22.18
0.8441	81.4	0.876	11.74
0.8190	91.4	0.834	6.28
0.7941	99.9	0.823	7.31

TABLE No. VI.—*The solubility of sodium acetate in aqueous solutions of ethyl alcohol at 25°*—Continued.

CALCULATED RESULTS.

The above results plotted on cross-section paper gave a curve from which the following figures were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$CH_3COONa+3H_2O$ per 100 grams.		Solvent to dissolve 1 gram $CH_3COONa+3H_2O$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.209	55.7	125.7	0.795
10.0	1.160	53.0	112.8	0.887
20.0	1.135	49.8	99.2	1.008
30.0	1.108	46.5	86.9	1.150
40.0	1.072	42.0	72.4	1.380
50.0	1.038	37.0	58.7	1.700
60.0	0.990	30.4	43.7	2.290
70.0	0.942	22.8	29.5	3.390
80.0	0.882	13.0	14.9	6.690
90.0	0.838	6.7	7.2	13.93
92.3	0.833	6.2	6.6	15.13
95.0	0.828	6.1	6.5	15.39
100.0	0.823	7.3	7.9	12.70

Solubility of zinc acetate in aqueous alcohol solutions.—As in the case of lead acetate, there is no method given in the Pharmacopœia for the quantitative analysis of this salt. The following method, based upon the determination of the zinc as oxide, was therefore used: A weighed sample of about 0.5 gram of the salt is dissolved in about 100 cubic centimeters of water and heated to the boiling point; dilute sodium carbonate solution (10 per cent) is then gradually added until the precipitation is complete. The heating continued for about one-half hour and the precipitate then filtered on a Gooch crucible, converted to oxide by igniting to bright redness, and weighed. The principal difficulty with the method is the tendency of the precipitate to adhere to the sides of the beaker and the almost unavoidable slight loss due to this cause. By great care, however, this source of error may be rendered practically negligible.

The sample of zinc acetate used for the following solubility determinations when analyzed by the above method gave an amount of zinc oxide corresponding to 102.4 per cent $Zn(C_2H_3O_2)_2+2H_2O$. This variation from the theoretical composition was no doubt due to loss of acetic acid, as has already been mentioned in connection with lead acetate. The odor of acetic acid was distinctly perceptible on opening the bottle containing the sample. It is possible that a sample of more nearly 100 per cent purity could have been prepared, but it is questionable whether the solubility results which it might have yielded would be of more interest than those here shown. The pharmacopœial purity rubric of 99.5 per cent for this salt is clearly unjustifiable, in view of the unstable character of the compound. It is quite probable that the sample used for the present investigation represents as good quality as the market may be expected to afford.

Only one series of determinations was made, but the time allowed for the attainment of saturation was five days. The saturated solutions were analyzed by precipitating the whole amount or an aliquot part by means of dilute sodium carbonate solution, as described above. The zinc oxide residues were analyzed in several cases by titration with standard acid and shown to be of the theoretical purity. The solid phase in contact with the alcoholic solutions of 96.3 and 99.9 weight per cent alcohol after the period of shaking was changed from its original crystalline character to the amorphous condition. Although the amount in either case was not sufficient for an analysis, there is little doubt that it consisted of the anhydrous salt, as was demonstrated for the amorphous lead acetate in contact with the strong alcohol solutions.

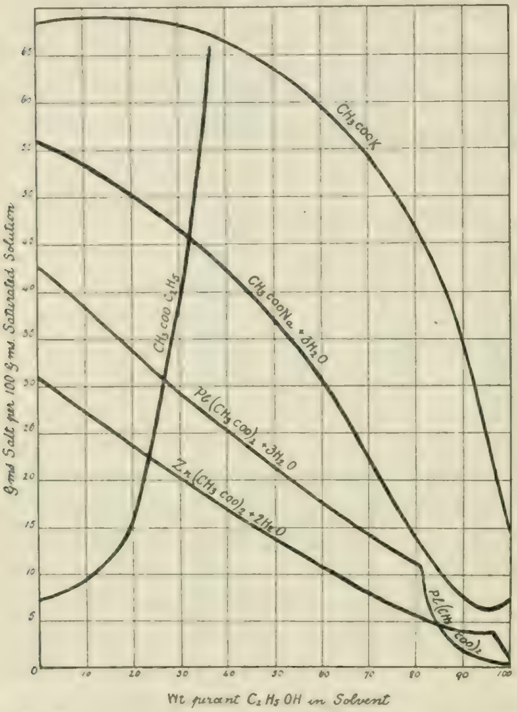

FIG. 1.—Curves showing the solubilities of the acetates in aqueous alcohol solutions at 25°.

TABLE NO. VII.—*The solubility of zinc acetate in aqueous solutions of ethyl alcohol at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	Acetate per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.168	30.78 $(C_2H_3O_2)_2Zn+2H_2O$
0.9856	8.9	1.132	27.64 $(C_2H_3O_2)_2Zn+2H_2O$
0.9545	32.0	1.048	19.78 $(C_2H_3O_2)_2Zn+2H_2O$
0.8718	70.2	0.878	7.90 $(C_2H_3O_2)_2Zn+2H_2O$
0.8190	91.4	0.832	4.16 $(C_2H_3O_2)_2Zn+2H_2O$
0.8048	96.3	0.822	3.87 $(C_2H_3O_2)_2Zn$
0.7941	99.9	0.796	1.18 $(C_2H_3O_2)_2Zn$

Table No. VII.—*The solubility of zinc acetate in aqueous solutions of ethyl alcohol at 25°*—Continued.

CALCULATED RESULTS.

The above figures plotted on cross-section paper gave a curve from which the following results were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$(C_2H_3O_2)_2Zn+2H_2O$ per 100 grams. Saturated solution.	$(C_2H_3O_2)_2Zn+2H_2O$ per 100 grams. Solvent.	Solvent to dissolve 1 gram $(C_2H_3O_2)_2Zn+2H_2O$.
		Grams.	*Grams.*	*Grams.*
0.0	1.168	30.80	44.5	2.25
10.0	1.127	27.20	37.4	2.67
20.0	1.090	23.70	31.1	3.22
30.0	1.055	20.40	25.6	3.90
40.0	1.015	17.00	20.5	4.88
50.0	0.970	13.80	16.0	6.25
60.0	0.920	10.60	11.9	8.42
70.0	0.880	7.80	8.5	11.80
80.0	0.850	5.50	5.8	17.20
90.0	0.830	4.20	4.4	22.80
92 3	0.827	4.10	4.3	23.40
95.0	0.825	4.00	4.2	24.60
100.0	0.796	[a] 1.18	[a] 1.2	[a] 83.75

[a] Solubility of the anhydrous salt.

BENZOIC ACID AND THE BENZOATES.

The solubility of benzoic acid in alcohol solutions.—The sample of benzoic acid used for the solubility determinations was analyzed by titration with standard alkali, using phenolphthaleine as indicator. Since the acid is only sparingly soluble in water, it is advisable to add enough alcohol to dissolve all of the sample previous to making the titration. It is to be noted, however, that since the ordinary alcohol is usually acid itself, it is necessary to previously neutralize that used for dissolving the benzoic acid. The titration gave average results showing a purity of practically 100 per cent. The melting-point determinations were within the limits quoted by the pharmacopœia.

The saturated solutions were analyzed by titrating aliquot portions after dilution, with 0.1 N NaOH. The results which were obtained are given in Table No. VIII and the solubility curve in Plate No. II.

Table No. VIII.—*Solubility of benzoic acid in aqueous ethyl alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent. Specific gravity at 15°.	Solvent. Per cent by weight of C_2H_5OH.	Specific gravity of saturated solution at 25°.	C_6H_5COOH per 100 grams saturated solution.
			Grams.
Dist. H_2O	0.0	1.000	0.367
0.9856	8.9	0.9865	0.581
0.9545	32.0	0.9574	4.677
0.9164	51.0	0.9464	17.800
0.8718	70.2	0.9404	30.070
0.8401	83.0	0.9311	35.050
0.8048	96.3	0.9128	36.510
0.7941	99.8	0.9093	36.910

TABLE NO. VIII.—*Solubility of benzoic acid in aqueous ethyl alcohol solutions at 25°*—Continued.

CALCULATED RESULTS.

The above results plotted on cross-section paper gave a curve from which the following figures were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	C_6H_5COOH per 100 grams.		Solvent to dissolve 1 gram C_6H_5-COOH.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.00	0.367	0.368	271.40
10.0	0.985	0.600	0.604	165.30
20.0	0.970	1.700	1.730	57.80
30.0	0.959	3.900	4.060	24.60
40.0	0.951	9.100	10.010	9.99
50.0	0.946	17.000	20.470	4.88
60.0	0.943	23.800	31.230	3.20
70.0	0.940	29.700	42.250	2.37
80.0	0.934	34.000	51.520	1.94
90.0	0.922	36.000	56.240	1.78
[a] 92.3	0.919	36.200	56.740	1.76
100.0	0.908	36.900	58.480	1.71

[a] U. S. Pharmacopœia strength.

The solubility of benzoic acid in organic solvents.—The solvents used for the determinations were obtained from different sources; the amyl alcohol, chloroform, ligroin, nitrobenzene, and toluene were the Kahlbaum products, the amyl acetate, benzene, cumene, and turpentine (double distilled) bore the Eimer and Amend label. A 5 cubic centimeter portion of each solvent was shaken with water and the acidity toward phenolphthaleine determined by titration with 0.1 N NaOH. Only the samples of naphtha and turpentine showed enough acidity to require a correction in the amount of alkali necessary for the saturated solution of the benzoic acid in these two solvents. No other quantitative tests were made of the purity of the solvents. The densities quoted in the table (No. IX) were taken for the most part from the second issue of Olsen's Chemical Annual and were used in the calculation of the grams of benzoic acid dissolved per 100 cubic centimeters of the several solvents.

The solubility determinations were made by rotating the glass-stoppered cylinders containing the solvents and an excess of the benzoic acid for four days at 25°. The clear saturated solutions were then weighed in a pycnometer, transferred to a glass-stoppered bottle with water, a few drops of phenolphthaleine added and the mixture titrated with 0.1 N NaOH solution.

TABLE No. IX.—*Solubility of benzoic acid in organic solvents at 25°.*

Solvent.	Specific gravity of solvent.	d_{25} of saturated solution.	C_6H_5COOH dissolved per—			Solvent to dissolve 1 gram C_6H_5COOH.
			100 grams saturated solution.	100 grams solvent.	100 c. c. solvent.	
			Grams.	*Grams.*	*Grams.*	*Grams.*
Amyl alcohol (iso)	$d_{20}=0.817$	0.875	24.45	32.37	26.44	3.09
Amyl acetate	$d_{20}=0.875$	0.912	18.24	22.00	19.26	4.54
Alcohol (abs.)	$d_{25}=0.785$	0.908	36.90	58.40	45.92	1.71
Benzene	$d_{25}=0.873$	0.897	10.90	12.23	10.76	8.17
Chloroform	$d_{22}=1.476$	1.456	13.15	15.14	22.35	6.61
Carbon tetrachloride	$d_{25}=1.591$	1.564	4.01	4.18	6.65	23.94
Carbon bisulphide	$d_{25}=1.258$	1.282	4.60	4.82	6.06	20.73
Cumene	$d_{20}=0.862$	0.906	7.91	8.59	7.41	11.04
Ether (abs.)	$d_{22}=0.711$	....	31.85	46.74	33.24	2.14
Ligroin	$d_{25}=0.714$	0.720	1.72	1.75	1.26	57.15
Naphtha	ca=0.720	0.730	2.48	2.65	1.91	37.70
Nitrobenzene	$d_{25}=1.204$	1.225	9.13	10.05	12.13	9.95
Toluene	$d_{15}=0.872$	0.884	9.46	10.69	9.33	9.57
Spirits of turpentine	$d_{20}=0.865$	0.859	4.84	5.09	4.40	19.66
Water	$d_4=1.000$	1.000	0.367	0.368	0.368	271.40
Xylene	$d_{25}=0.861$	0.877	8.85	9.71	8.52	9.71

The solubility of ammonium benzoate in aqueous alcohol solutions.—The sample of ammonium benzoate used for the solubility determinations was recrystallized from warm alcohol and washed with ether. Analyses showed it to contain 99.8 per cent $C_6H_5COONH_4$. In some cases unrecrystallized material of approximately 98.8 per cent purity was used. The analytical details involved in the determination of the purity of samples of ammonium benzoate have already been described in a paper from this laboratory entitled "Pharmacopœial Tests for Ammonium Benzoate."[a] It was shown that the most satisfactory method of analysis is the determination of the ammonia liberated by caustic alkali. The melting or decomposition point is of no value whatever in judging the purity of a sample of the salt. Furthermore, the litmus paper test is inadequate for showing the presence of less than 10 per cent of benzoic acid.

The analysis of the salt and of the saturated solution was made by distillation of the ammonia after adding an excess of caustic alkali. The distilled ammonia was titrated in the usual way, and in addition the amount of alkali required to liberate the ammonia was determined by titrating the portion of the liquid remaining in the distilling flask. The benzoate calculated from the two titrations agreed only fairly well, therefore the values obtained from the ammonia titrations were used for the solubility determinations reported in the accompanying table, since the results calculated from the alkali neutralized in the distilling flask are subject to the error arising from the solubility of the glass and from inaccuracies of the indicator.

The periods of shaking the tubes were from one to three days. The curve is interesting in that it is almost horizontal in the region of dilute alcoholic solvents. Thus alcohol solutions between 5 and 40 per cent strength dissolve practically the same amount of ammonium benzoate. The figure for the solubility in water, as given by the Pharmacopœia, is evidently incorrect, the actual solubility being more than twice as great as stated. The result given for U. S. P. alcohol, on the other hand, is very near the figure found in the present investigation.

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[a] Seidell and Menge, Am. Journ. of Pharmacy, **82**, 12–30, 1910.

TABLE NO. X.—*Solubility of ammonium benzoate in aqueous alcohol solutions at 25° C.*

EXPERIMENTAL RESULTS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_6H_5COONH_4$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.043	18.6
0.9856	8.9	1.029	18.0
0.9545	32.0	0.993	18.1
0.9164	51.0	0.954	16.9
0.8718	70.2	0.900	12.1
0.8190	91.4	0.825	3.8
0.8048	96.3	0.808	2.5
0.7941	99.9	0.796	1.6

CALCULATED RESULTS.

The above results, plotted on cross-section paper, gave a curve from which the following figures were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$C_6H_5COONH_4$ per 100 grams.		Solvent to dissolve 1 gram $C_6H_5COONH_4$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.043	18.6	22.8	4.38
10.0	1.027	18.0	22.0	4.55
20.0	1.012	18.0	22.0	4.55
30.0	0.997	18.1	22.1	4.53
40.0	0.979	18.0	22.0	4.55
50.0	0.956	17.0	20.5	4.88
60.0	0.930	15.0	17.6	5.67
70.0	0.901	12.2	13.9	7.20
80.0	0.864	8.3	9.1	11.05
90.0	0.828	4.2	4.4	22.81
[a] 92.3	0.819	3.4	3.5	28.41
95.0	0.810	2.7	2.8	36.03
100.0	0.796	1.6	1.6	61.15

[a] U. S. Pharmacopœia strength.

The solubility of lithium benzoate in aqueous alcohol solutions.—The sample of lithium benzoate was in the form of a very fine white powder. The aqueous solution did not react alkaline to litmus paper, but was, on the other hand, acid towards phenolphthaleine. Two grams of the sample dissolved in about 50 cubic centimeters of water required 1.4 cubic centimeters 0.1 N NaOH for neutralization, indicator phenolphthaleine. Therefore indicating the presence of 0.85

per cent free benzoic acid. A determination of the lithium by the pharmacopœial method gave an average amount corresponding to 99.2 per cent lithium benzoate. The sample was therefore well within the limit of purity required by the Pharmacopœia, viz., 98.5 per cent. It appears that the pharmacopœial statement regarding the reaction of lithium benzoate to indicators should be changed to prescribe neutrality toward litmus (or better the omission of reference to litmus) and not exceeding a definite acidity toward phenolphthaleine. The additional pharmacopœial tests applied to the present sample gave further evidence of its satisfactory quality. The benzoic acid obtained from it gave a melting point of 121° (cor.) and the lithium chloride dissolved practically completely in amyl alcohol and contained Cl corresponding to a purity of 99.7 per cent. Although for some reasons it might have been better to have used a sample of more nearly 100 per cent purity than the present one, it is probable that the small percentage of free benzoic acid could not appreciably affect the solubility values obtained. In preparing the saturated solutions, an excess of salt was added to each solvent in the usual way and the tubes rotated at 25° for two days. The weighed portions of the saturated solutions were transferred to weighing bottles and evaporated in a vacuum desiccator containing concentrated sulphuric acid. No especial attempt was made to carry the desiccation to the end, but only until the residues were sensibly dry. Portions of each were then removed and the lithium determined as sulphate by fusion with ammonium sulphate according to the method of the pharmacopœia. The weight of anhydrous benzoate in each residue was then calculated from the lithium sulphate found. The curve which was obtained (see Plate No. II) is perfectly regular, and only one series of determinations made after a two-day period of shaking was deemed necessary. It will be noted that a maximum solubility occurs at about 15 per cent alcohol. In the case of the ammonium benzoate curve a slight minimum exists at about this concentration of solvent.

TABLE No. XI.—*Solubility of lithium benzoate in aqueous alcohol solutions at 25° C.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	C_6H_5COOLi per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O.	0.0	1.103	27.64
0.9856	8.9	1.090	28.52
0.9545	32.0	1.047	27.79
0.9164	51.0	0.999	23.46
0.8718	70.2	0.931	15.23
0.8190	91.4	0.838	5.98
0.8048	96.2	0.815	4.02
0.7941	99.9	0.799	2.61

TABLE NO. XI.—*Solubility of lithium benzoate in aqueous alcohol solutions at 25° C.*—Continued.

CALCULATED RESULTS.

The above results plotted on cross section paper gave a curve from which the following figures were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	C_6H_5COOLi per 100 grams.		Solvent to dissolve 1 gram C_6H_5COOLi.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.103	27.64	38.2	2.62
10.0	1.088	28.60	40.1	2.50
20.0	1.072	28.50	39.9	2.51
30.0	1.052	27.80	38.5	2.60
40.0	1.030	26.20	35.5	2.82
50.0	1.003	23.60	30.9	3.24
60.0	0.970	19.80	24.7	4.05
70.0	0.932	15.40	18.2	5.49
80.0	0.890	10.70	11.9	8.35
90.0	0.847	6.40	6.8	14.62
[a] 92.3	0.835	5.50	5.8	17.18
95.0	0.823	4.50	4.7	21.22
100.0	0.799	2.60	2.7	37.46

[a] U. S. Pharmacopœia strength.

Solubility of sodium benzoate in aqueous alcohol solutions.—Several difficulties were encountered while investigating the solubility of this salt. In the first place, the purification of the material by recrystallization could not be accomplished by any means at command. The solubility of the salt in water is so great that the saturated solution is rather viscous, and on standing shows no tendency towards crystallization. The liquid climbs up the sides of the vessel and deposits a crust as the solvent evaporates. A hot saturated aqueous solution yields a crust on the surface, or if sufficiently concentrated will form a solid mass upon cooling. Alcohol, ether, and various mixtures of these with water were tried without success. In all cases the salt deposited at the surface as the solvent evaporated and no crystals appeared in the solution.

In analyzing the salt by the pharmacopœial method, which requires that the sample be ignited and the solution of the residue titrated with standard acid, using methyl orange as the indicator, it was found that concordant results could not be obtained unless the charred residue remaining after the incineration and extraction with hot water be ignited and the second residue dissolved in water and added to the first extract. The several determinations showing the additional amounts of the salt recovered by the second ignition are as follows:

Weight of sample.	0.5 N HCl required—		Calc. per cent C_6H_5COONa.	
	After first incineration.	After second incineration.	U. S. P.	Modified.
Gram.	*c. c.*	*c. c.*		
1.0	13.15	13.50	94.7	97.4
1.0	12.70	13.55	91.45	97.6
1.0	12.90	13.40	92.9	96.5
1.0	12.60	13.40	90.7	96.5

The method as modified was therefore used in all cases for the analysis of the samples and of the residues remaining after evaporation of the saturated solutions obtained in the solubility determinations. An attempt was made to prepare a pure sample of sodium benzoate by fractionating a hot aqueous solution of the salt as follows: The solution was evaporated over a flame until a crust just began to form; it was then cooled somewhat and the separated salt removed, dried on filter paper, and finally in a vacuum desiccator containing concentrated H_2SO_4. The liquor from this fraction was then evaporated until a second crust was formed and then cooled as before and the new fraction removed and dried. The final liquor was evaporated far enough so that it solidified on cooling. The original material and the three products were thoroughly dried in a vacuum desiccator containing conc. H_2SO_4 and analyzed. The results were, respectively, 97.5, 98.1, 98.4, and 98.4 per cent, showing the process to be only partially successful. It seems probable that the differences between the results found and 100 per cent are due to adhering water which can not be removed by vacuum desiccation. It is questionable whether the pharmacopœial requirement of 99 per cent C_6H_5COONa is a reasonable limit. Of two purchased samples, claimed to be of U.S.P. purity, one gave 97.5 and the other 96.5 per cent C_6H_5COONa upon analysis. The efforts made in the laboratory to prepare a product of the pharmacopœial purity from one of these samples was unsuccessful.

The material used for the solubility determinations was the product containing by analysis 97.5 per cent C_6H_5COONa. The determination of the free benzoic acid in this sample by titration with standard alkali showed 0.36 per cent. It was found that in preparing the saturated solutions for the solubility determinations it was advisable to have only a very small excess of the solid, since the undissolved salt becomes apparently gelatinous, and may form an almost solid mass from which it is impossible to remove enough clear saturated solution for analysis. Even with only a slight excess of the salt considerable time is required for the suspended solid to subside. This happens in both the aqueous and alcoholic solutions. After considerable difficulty the tubes were prepared with a satisfactory excess of the solid in each and rotated at 25° two days; in the case of one duplicate determination the time of rotation was extended to three days without changing the figure obtained in the two-day period.

The saturated solutions were weighed in the pycnometer as usual and transferred to weighed weighing bottles and evaporated in a vacuum desiccator containing conc. H_2SO_4 until no further loss in weight occurred. The residues were then dissolved in water and the whole, or an aliquot portion of each solution, evaporated to dryness in a platinum dish and incinerated, the alkali being determined by titration, as already described. The values calculated from the analysis were in all cases, except with the solvents of highest alcoholic content, 97 to 98 per cent of the weight of dried residue obtained after the desiccation, thus lending weight to the view that the original impurity in the sample was nothing else than adhering water and a slight amount of free benzoic acid. Otherwise it would be expected that the residues from the solubility determinations would show a different degree of purity than the original material. The results of the determinations are shown in Table No. XII and the curve of the solubility in figure 2.

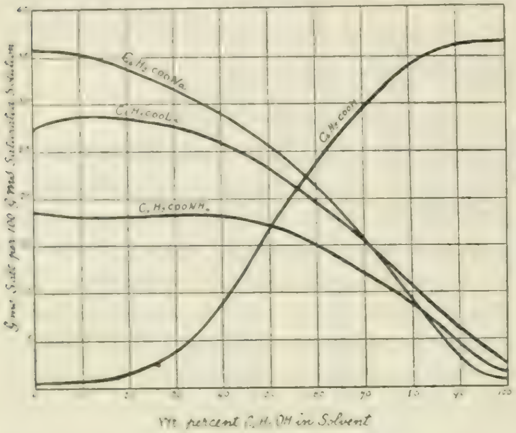

FIG. 2.—Curves showing the solubilities of benzoic acid and the benzoates in aqueous alcohol solutions at 25°.

TABLE No. XII. *Solubility of sodium benzoate in aqueous alcohol solutions at 25° C.*

EXPERIMENTAL RESULTS.

Solvent.		Specific gravity of saturated solution at 25°.	C_6H_5COONa per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O.	0.0	1.155	35.99
0.9856	8.9	1.137	35.53
0.9545	32.0	1.082	31.74
0.9164	51.0	1.016	25.43
0.8718	70.2	0.927	15.25
0.8190	91.4	0.825	2.25
0.8048	96.3	0.807	1.05
0.7941	99.9	0.795	0.58

TABLE No. XII.—*Solubility of sodium benzoate in aqueous alcohol solutions at 25°*—Continued.

CALCULATED RESULTS.

The above results plotted on cross-section paper gave a curve from which the following figures were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	C_6H_5COONa per 100 grams.		Solvent to dissolve 1 gram C_6H_5COONa.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.155	36.0	56.24	1.78
10.0	1.132	35.3	54.39	1.88
20.0	1.110	33.7	50.83	1.97
30.0	1.086	31.5	45.98	2.18
40.0	1.055	28.9	40.64	2.46
50.0	1.020	25.6	34.41	2.91
60.0	0.975	21.3	27.07	3.69
70.0	0.927	15.4	18.20	5.49
80.0	0.877	8.8	9.65	10.36
90.0	0.831	2.8	2.88	34.71
[a] 92.3	0.822	2.0	2.04	49.00
95.0	0.812	1.3	1.32	75.93
100.0	0.795	0.6	0.60	165.70

[a] U. S. Pharmacopœia strength.

CAMPHORIC ACID.

The solubility of camphoric acid in aqueous alcohol solutions at 25°.—The material used for the solubility determinations was pure white crystalline powder, and when analyzed by titrating weighed portions with standard alkali, using phenolphthalein as indicator, gave results indicating a purity of practically 100 per cent. The sample was dextro rotatory. The melting point was found to be 184° to 187° (cor). Two series of solubility determinations were made; the time allowed for the attainment of saturation was four days in one case and six in the other. The saturated solutions were analyzed by evaporating and drying the residue to constant weight at 100°. A blank test made at the same time showed that the original sample lost practically nothing when dried under the same conditions.

The curve drawn from the results obtained is interesting in that a maximum point occurs at about 85 weight per cent alcohol. It is therefore seen that although the solubility of camphoric acid increases very rapidly with increase of alcoholic strength through the greater part of its length, beyond a concentration of about 85 weight per cent alcohol a diminution of solubility occurs and less camphoric acid is dissolved by absolute than by alcohol of the U. S. Pharmacopœia strength.

TABLE No. XIII.—*Solubility of camphoric acid in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_8H_{14}(COOH)_2$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
1.000	0.0	1.000	0.754
0.9856	8.9	1.000	1.239
0.9545	32.0	1.000	16.29
0.9164	51.0	1.000	38.61
0.8718	70.2	1.000	48.95
0.8401	83.0	0.985	51.46
0.8189	91.4	0.980	51.13
0.8048	96.3	0.970	50.37
0.7941	99.8	0.960	50.13

CALCULATED DETERMINATIONS.

The above results plotted on cross section paper gave a curve from which the following figures were read or calculated.

Per cent by weight of alcohol in solvent.	Specific gravity of saturated solution at 25°.	$C_8H_{14}(COOH)_2$ per 100 grams.		Solvent to dissolve 1 gram $C_8H_{14}(COOH)_2$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.000	0.754	0.76	131.60
10.0	1.000	1.6	1.63	61.50
20.0	1.000	6.3	6.72	14.87
30.0	1.000	14.0	16.28	6.14
40.0	1.000	26.0	35.14	2.84
50.0	1.000	38.0	61.30	1.63
60.0	1.000	45.0	81.81	1.22
70.0	1.000	49.0	96.12	1.04
80.0	0.995	51.2	104.9	0.95
85.0	0.987	51.6	106.6	0.94
90.0	0.980	51.4	105.8	0.945
[a] 92.3	0.977	51.1	104.5	0.957
95.0	0.974	50.8	103.2	0.968
100.0	0.960	50.1	100.4	0.996

[a] U. S. Pharmacopœia strength.

The statements of the solubility of camphoric acid as quoted in the literature are very variable. For water they range between 1 part acid per 160 parts water at 12° (Beilstein) to 1 part in 125 water at 25° (U. S. P.). The present results indicate that the correct figure is 1 part in 131.6 parts water at 25°. For alcohol even wider variations are to be found. Thus, Beilstein gives 1 part in 0.89 parts alcohol at 15.5° and Hager[a] quotes 1 part in 1.3 parts alcohol. This divergence probably accounts for the fact that no figure is quoted by the U. S. Pharmacopœia and only the description "readily soluble in alcohol" is given. The present results show that 1 part camphoric acid requires 0.957 grams of U. S. Pharmacopœia alcohol (of 92.3 weight per cent) for solution.

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[a] Handbuch der Pharmaceutischen Praxis. (1903.)

The complete results which were obtained are given in Table No. XIII, and the curve shown in figure 4.

Solubility of camphoric acid in organic solvents.—In addition to the solubility determinations in aqueous alcohol solutions a series was made in a large number of organic solvents. The solvents were described in connection with benzoic acid (p. 24). The time of rotation was two days; the dissolved acid was determined by titration with 0.1 N NaOH or N NaOH in the cases where much acid was present. The results are given in Table No. XIV. It will be noted that with the exception of absolute alcohol, ether and amyl alcohol are the only solvents which dissolve large amounts of camphoric acid. The result for ether is almost twice that quoted by Hager,[a] viz, 1 part in 1.8 parts; that is, 55 grams per 100 grams ether, and likewise the present figure for chloroform is higher than the quoted value of 1 part per 1,000. The present result for carbon bisulphide confirms the statement given in Beilstein in regard to the practical insolubility of camphoric acid in this solvent.

TABLE NO. XIV.—*Solubility of camphoric acid in organic solvents at 25°.*

Solvent.	Specific gravity of solvent.	Specific gravity of saturated solution at 25°.	$C_8H_{14}(COOH)_2$ dissolved per—			Solvent to dissolve 1 gram $C_8H_{14}(COOH)_2$.
			100 grams saturated solution.	100 grams solvent.	100 c. c. solvent.	
			Grams.	*Grams.*	*Grams.*	*Grams.*
Amyl alcohol (Iso)	$d_{20}=0.817$	0.907	33.320	50.000	40.800	2.0
Alcohol (abs.)	$d_{25}=0.785$	0.960	50.100	100.400	78.800	0.996
Benzene	$d_{25}=0.873$	0.873	0.008	0.008	0.007	12,500.0
Carbon bisulphide	$d_{25}=1.258$	1.258	0.020	0.020	0.025	5,000.0
Chloroform	$d_{22}=1.476$	....	0.153	0.153	0.230	652.5
Cumene	$d_{20}=0.863$	0.890	0.197	0.197	0.170	506.5
Ether (abs.)	$d_{22}=0.711$	0.922	47.750	91.400	65.000	1.094
Ligroin	$d_{25}=0.714$	0.714	0.007	0.007	0.005	14,290.0
Nitro benzene	$d_{25}=1.205$	1.198(?)	0.500	0.500	0.600	199.0
Spirits of turpentine	$d_{20}=0.865$	0.852	1.710	1.740	1.500	57.6
Toluene	$d_{25}=0.864$	0.862	0.151	0.150	0.130	661.2
Water	$d_4=1.000$	1.000	0.754	0.760	0.760	131.6
Xylene	$d_{25}=0.861$	0.859	0.233	0.230	0.200	427.9

CITRIC ACID AND THE CITRATES.

Solubility of citric acid in aqueous alcohol solutions.—The material used for the following solubility determinations was in the form of rather large clear crystals. The analysis by titration with normal alkali using phenolphthaleine as indicator gave an average of 100.1 per cent $(CH_2)_2C(OH)(COOH)_3 + H_2O$. For the first series of solubility determinations an excess of the original sample of citric acid was added to each of the alcoholic solvents and the tubes rotated three days. After this time it was observed that the solid phase in the tubes of higher alcoholic concentration had changed from its original crystalline form to a more or less opaque powder, indicating that dehydration had occurred at about 70 to 80 weight per cent alcohol. The clear saturated solutions were withdrawn as usual and the dissolved citric acid determined by titration with normal alkali. The results calculated to the hydrated citric acid gave a curve with a slight but unmistakable irregularity at the alcoholic concentration corresponding to the change of solid phase. When the figures for the alcoholic solutions containing the opaque solid phase were calculated to the anhydrous acid the curve then lay below and almost parallel to the curve for the hydrated acid and gave no indication of there being a point of intersection of the two, as would be expected if the solid phase had changed completely. The dehydration by the stronger alcoholic solvents therefore appeared incomplete, and it was decided to prepare a quantity of the anhydrous acid by drying and use it as the solid phase for a series of determinations. The dehydration of the sample was effected by drying in an oven at approximately 85° for about twenty-four hours. The analysis showed the material thus dried to contain 98.9 per cent anhydrous citric acid. All of the determinations made with the dehydrated acid as solid phase except the two in which water alone and 8.9 per cent alcohol were the solvents gave a perfectly regular curve lying some distance below the values for the anhydrous acid calculated from the determinations made by starting with the hydrated acid. In the case of the water and 8.9 per cent alcohol the anhydrous acid was converted to the crystalline compound and the results agreed exactly with the determinations made with hydrated citric acid as the solid phase. By calculating all of the results obtained with the hydrated citric acid to anhydrous acid a curve was obtained which lay below the curve for the anhydrous acid in the dilute alcohol solutions, and above the latter in the more concentrated alcohol solutions. It may be concluded, therefore, that the tendency for the hydrated form of citric acid to be converted to the anhydrous or vice versa in aqueous alcohol solutions is very slight, and that whatever form of the acid is used as the solid phase, the change to the other form takes place very gradually. The peculiarity in connection with the curves showing the solubility of the hydrated and anhydrous forms of citric acid is that they lie so nearly parallel that there is no intersection corresponding to a true transition point as has been shown in the case of the zinc and lead acetate curves.

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[a] Handbuch der Pharmaceutischen Praxis. (1903.)

Table No. XV.—*The solubility of citric acid in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$(CH_2)_2COH(COOH)_3+H_2O$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist H_2O	0.0	1.311	67.5
0.9854	8.9	1.302	66.9
0.9545	32.0	1.270	65.3
0.9164	51.0	1.236	63.2
0.8718	70.2	1.193	[a] 60.7
0.8441	81.5	1.156	[a] 57.6
0.8190	91.4	1.121	[a] 54.1
0.7941	99.9	1.069	[a] 49.9

[a] Solid phase changed more or less completely during the period of saturation from crystalline to opaque appearance.

CALCULATED RESULTS.

The above results plotted on cross-section paper gave a curve from which the following figures were obtained:

Per cent by Weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$(CH_2)_2COH(COOH)_3+H_2O$ per 100 grams.		Solvent to dissolve 1 gram $(CH_2)_2COH(COOH)_3+H_2O$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.311	67.5	207.7	0.481
10.0	1.300	66.8	201.2	0.497
20.0	1.286	66.0	194.1	0.515
30.0	1.272	65.3	188.2	0.531
40.0	1.257	64.3	180.1	0.555
50.0	1.237	63.3	172.5	0.580
60.0	1.216	62.0	163.1	0.613
70.0	1.192	60.8	155.1	0.645
80.0	1.163	58.1	138.7	0.721
90.0	1.125	54.7	120.8	0.828
92.3	1.114	53.7	116.0	0.862
95.0	1.100	52.4	110.1	0.908
100.0	1.068	49.8	99.2	1.008

Table No. XVI. *Solubility of anhydrous citric acid in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

[Solid phase—Anhydrous salt.]

Solvent.		Specific gravity of saturated solution at 25°.	$(CH_2)_2COH(COOH)_3$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
0.9545	32.0	1.268	60.6
0.9164	51.0	1.216	57.1
0.8718	70.2	1.160	51.9
0.8441	81.5	1.115	47.9
0.8190	91.4	1.057	43.0
0.7941	99.9	1.010	38.4

Table No. XVI.—*Solubility of anhydrous citric acid in aqueous alcohol solutions at 25°*—Continued.

CALCULATED RESULTS.

Per cent by Weight of C_2H_5OH in solvent	Specific gravity of saturated solution at 25°.	$(CH_2)_2COH(COOH)_3$ per 100 grams saturated solution.
		Grams.
20	1.297	62.3
40	1.246	59.0
60	1.190	54.8
70	1.160	52.2
80	1.120	48.5
90	1.065	43.7
100	1.010	38.3

The analytical results upon the ordinary and anhydrous forms of citric acid are given in Tables Nos. XV and XVI and the curves shown in figure 3. It will be seen that the present values differ considerably from those quoted by the U. S. Pharmacopœia, which in terms of grams of citric acid per 100 grams of water and of alcohol would be 185 and 64.5, respectively, instead of 207.7 and 116.0 as given in Table No. XV. The values quoted by other reference books also differ considerably from those here shown, but since they probably refer to a temperature of 15° a strict comparison can not be made. It should be mentioned, however, that the differences can not be accounted for entirely on the basis of temperature, since a few results seem to show that the solubility of citric acid increases comparatively little with temperature, while most of the values to be found for the solubility in cold water and alcohol are only a little more than one-half the quantities indicated by the present determinations, which were made at 25° C.

Solubility of citric acid in organic solvents.—The determinations were made, as already described, with the same organic solvents used for benzoic and camphoric acids. Both the hydrated and anhydrous citric acid were used, and it is seen that the latter is the less soluble of the two in all cases. The results are given in Table XVII. An examination of the literature shows that in addition to water and alcohol, results are given for none of the solvents included in the table, except ether. The results quoted for ether are quite variable; thus 100 parts of ether dissolve, according to the U. S. Pharmacopœia, 5.5 parts of citric acid, 2 parts according to Hager, and 9.1 parts are reported by Beilstein. These variations are easily explained by the fact that the solubility of citric acid is very great in water and in alcohol, and therefore the presence of either of these solvents in the ether used for the determinations would account for the different values reported. A few results obtained by me with ether from a can which had been opened for some time illustrated this point very forcibly. The values were from about 30 to 80 per cent higher than those given in the tables for absolute ether. The figures are not given herewith, however, since the actual composition of the ether was not known, and they therefore have no especial interest.

TABLE No. XVII.—*Solubility of citric acid in several organic solvents at 25°.*

SOLUBILITY OF HYDRATED CITRIC ACID.

Solvent.	Specific gravity of solvent.	d_{25} of saturated solution.	$(CH_2)_2C(OH)(COOH)_3+H_2O$ dissolved per—			Solvent to dissolve 1 gram $(CH_2)_2C(OH)(COOH)_3+H_2O$.
			100 grams solution.	100 grams solvent.	100 c. c. solvent.	
			Grams.	*Grams.*	*Grams.*	*Grams.*
Amyl acetate	$d_{20}=0.8750$	0.8917	5.980	6.360	5.67	15.690
Amyl alcohol (iso)	$d_{20}=0.8170$	0.8774	15.430	18.240	16.01	5.480
Ethyl alcohol (abs.)	$d_{15}=0.7940$	1.0690	49.800	99.200	78.75	1.008
Ethyl acetate	$d_{25}=0.8915$	0.9175	5.276	5.570	5.11	17.950
Ether (abs.)	$d_{22}=0.7110$	0.7228	2.174	2.223	1.60	45.000
Chloroform	$d_{22}=1.4760$	1.4850	0.007	0.007	0.01	14,290.0

The amounts of citric acid dissolved by the following solvents were too small for estimation:

Benzene, carbon bisulphide, carbon tetrachloride, toluene.

SOLUBILITY OF ANHYDROUS CITRIC ACID.

Solvent.	Specific gravity of solvent.	d_{25} of saturated solution.	$(CH_2)_2C(OH)(COOH)_3$ dissolved per—			Solvent to dissolve 1 gram $(CH_2)_2C(OH)(COOH)_3$.
			100 grams solution.	100 grams solvent.	100 c. c. solvent.	
			Grams.	*Grams.*	*Grams.*	*Grams.*
Amyl acetate	$d_{20}=0.875$	0.8861	4.22	4.41	3.900	22.70
Ethyl alcohol (abs.)	$d_{15}=0.794$	1.0100	38.40	62.20	49.400	1.61
Ether (abs.)	$d_{22}=0.711$	0.7160	1.05	1.06	0.757	94.50
Chloroform	$d_{22}=1.476$	1.4880	None.	None.	None.	....

Solubility of bismuth citrate in aqueous alcohol solutions.—Although the U. S. Pharmacopœia gives the ordinary formula for neutral bismuth citrate, the purity rubric requires simply that the preparation contain 56 to 58 per cent bismuth oxide. The theoretical percentage of bismuth oxide according to the formula is 58.43, and therefore the variation allowed by the pharmacopœia corresponds to a range of purity from 95.94 to 99.24 per cent. The determination of the bismuth oxide according to the pharmacopœial method offers considerable difficulties. The original ignition of the sample is easily accomplished, but the subsequent evaporation of the excess of nitric acid which must be added is attended by creeping of the liquid over the edge of the crucible; furthermore, just as the last of the excess of nitric acid is being driven off a slight explosion of the nitrate usually occurs, due to the presence of undecomposed carbon, and therefore leads to a probable loss of a portion of the residue. A determination of the bismuth in the sample used for the solubility experiments gave 0.553

gram Bi_2O_3 per 1.0 gram of sample, which is slightly below the pharmacopœial requirement, and corresponds to a purity of 94.62 per cent. This result may, however, be low, since it is not certain that no loss occurred during the determination. With a compound having the more or less uncertain composition of the present one, no very precise solubility results could be expected. The following determinations therefore represent only approximate values. The time of shaking was four days at 25° C. Three tubes were prepared, the solvents being water, 51 and 91.4 weight per cents alcohol. The saturated solutions were evaporated to dryness in weighing bottles and the residues weighed. The following results were obtained:

Per cent by weight of C_2H_5OH in solvent.	$BiC_6H_5O_7$ per 100 grams saturated solution.
	Grams.
0.0	0.011
51.0	0.041
91.4	0.005

Solubility of bismuth and ammonium citrate in aqueous alcohol.—A formula is not given by the pharmacopœia for this compound, and the requirements of bismuth content covers a wider range than in the case of bismuth citrate. The allowable content according to the pharmacopœial purity rubric is 46 to 50 per cent bismuth oxide. The same sources of error are present in the quantitative method of determination of the bismuth as have already been pointed out for the bismuth citrate with the further difficulty due to decrepitation during the initial ignition. The content of bismuth oxide in the sample used for the solubility determinations was found to be 0.475 gram, Bi_2O_3 per 1.0 gram, thus well within the limit given by the pharmacopœia. The following solubility results are therefore probably fairly representative of the product as described by the pharmacopœia. For these determinations the tubes were rotated at 25° four days; the weighed saturated solutions were transferred to porcelain crucibles, evaporated to dryness, and the residue converted to bismuth oxide and weighed. The weight of the original material was calculated by dividing by the percentage of Bi_2O_3 found in the sample used for the determinations. The results were as follows:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	Bi and NH_4 citrate per 100 grams saturated solution.
		Grams.
0.0	1.25	22.25
51.0	0.92	1.34
91.4	0.81	None.

The only statement in regard to the solubility of bismuth and ammonium citrate given in the pharmacopœia is to the effect that it is very soluble in water and sparingly soluble in alcohol. The present results therefore will serve to give a somewhat better idea in regard to solubility of this substance in these two solvents than could have previously been obtained.

Solubility of lithium citrate in aqueous alcohol solutions.—The pharmacopœial quantitative method of analysis of this compound requires that the weighed sample be dried at 150° and then cautiously ignited, after which the charred residue is converted to lithium sulphate by repeated additions of concentrated sulphuric acid and careful ignitions. The drying at 150° appears to be unnecessary, since the water of crystallization can be satisfactorily removed by heating with care over the free flame. There appears some advantage in adding anhydrous ammonium sulphate and mixing after the dehydration and before proceeding to the ignition. The use of ammonium sulphate does not, however, obviate the necessity for adding concentrated sulphuric acid repeatedly to obtain the required white residue. In attempting to determine the water of crystallization of a sample by drying at 150° it was found that the loss of weight was very gradual, so that even after a total of about fifteen hours' drying with the temperature as high as 175° constant weight had not been obtained. The loss of moisture corresponded to 23.4 per cent instead of the theoretical 25.0 per cent for 4 molecules of water of crystallization. The determination of the lithium in this sample as above outlined gave results corresponding to 98.4 per cent $(CH_2)_2COH(COOLi)_3+4H_2O$. The acidity calculated as citric acid corresponded to 0.35 per cent. Although this result shows that the sample is practically up to the pharmacopœial requirement of 98.5 per cent, it was thought desirable to prepare material of better quality if possible. This was done by recrystallization from alcohol of about 50 per cent strength. The hot saturated alcoholic solution showed some tendency to separate into two layers, but on standing crystals began to separate from the lower portion of the solution, and on stirring the mixture was apparently uniform. Upon a further cooling a good crop of crystals was obtained. They were washed once with strong alcohol, dried in an ordinary desiccator over night, and for a few minutes at 40° to 50°. The analysis gave results corresponding to a purity of 99.3 per cent $(CH_2)_2COH(COOLi)_3+4H_2O$.

The solubility determinations were made with both the original and recrystallized samples, but no differences in the values obtained could be definitely ascribed to a difference of purity of the two samples. Three series of determinations were made and the time of shaking varied from two to three days. All of the points except one, viz, that for 17.0 weight per cent alcohol fell on the very regular curve (see fig. 3) drawn through them. It is probable that in this one case the discrepancy was due to some error for which I am unable to account. The value is given in Table No. XVIII, however, for the sake of completeness, but it is not used in obtaining the interpolated results for regular intervals of alcoholic strengths.

The analyses of the saturated solutions were made by evaporating in weighing bottles and drying the residues to practically constant weight at 170° to 180°. Portions of the residues were then used for lithium determinations by igniting and converting to lithium sulphate, and these results served as checks upon the thoroughness of the drying process.

In comparison with the present results it will be seen that the figures quoted by the pharmacopœia are very much too low.

TABLE No. XVIII.—*Solubility of lithium citrate in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$(CH_2)_2COH(COOLi)_3 + 4H_2O$ per 100 grams saturated solution.
d_{16}.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.2160	42.70
0.9856	8.9	1.1570	33.50
[a] (0.9752	17.0	1.1150	29.00)
0.9628	26.4	1.0430	18.50
0.9359	41.7	0.9692	7.90
0.9164	51.0	0.9296	4.40
0.8718	70.2	0.8674	0.54
0.8234	89.6	0.8166	0.06
0.7941	99.9	0.7883	0.02

[a] This determination not used in plotting the curve.

CALCULATED RESULTS.

The above figures plotted on cross-section paper gave a curve from which the following results were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$(CH_2)_2COH(COOLi)_3 + 4H_2O$ per 100 grams.		Solvent to dissolve 1 gram $(CH_2)_2COH(COOLi)_3 + 4H_2O$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0	1.216	42.70	74.50	1.34
10	1.150	33.00	49.30	2.03
20	1.083	24.30	32.10	3.12
30	1.025	15.80	18.80	5.33
40	0.976	8.80	9.65	10.36
50	0.933	4.70	4.93	20.30
60	0.897	2.20	2.25	44.50
70	0.867	0.60	0.60	165.80
80	0.838	0.30	0.30	332.40
100	0.788	0.02	0.02	4,999.00

Solubility of potassium citrate in aqueous alcohol.—This salt belongs to that group of compounds which has the power of separating mixtures of alcohol and water into layers consisting of more or less pure alcohol as the upper and the aqueous salt solution as the lower. A systematic search for the members of this group of compounds was made some years ago by Linebarger,[a] and although a number were found no experiments were made with citrates, and therefore the fact that postassium citrate possesses this power of forming layers with aqueous alcohol solutions was overlooked.

The sample of potassium citrate used for the solubility determinations described in the following pages was purified by recrystallization from the hot aqueous solution, washing with absolute alcohol and ether and drying in an air bath at 130°–150° for five hours. The statement of the pharmacopœia that the salt begins to lose water at as low as 100° is evidently incorrect, since the present sample retained its water of crystallization even when heated to 150°. The analysis by igniting and titrating the residue, as described under sodium benzoate (p. —), gave 98.6 per cent $(CH_2)_2COH(COOK)_3 + H_2O$. The acidity calculated as citric acid corresponded to 0.94 per cent.

The solubility determinations were made by adding an excess of the salt to about 10 to 15 cubic-centimeter portions of the several alcohol solutions of known strengths and rotating the tubes for five days at 25°. After this time they were each removed in a beaker filled with the water of the constant temperature bath and as much as possible of each layer drawn into a pycnometer by means of the suction siphon described in the first part of this bulletin (p. 11). In the case of several of the solutions enough of both layers to fill the pycnometer could not be obtained, and therefore the specific gravity could not be calculated. A dotted line in the Table No. XIX indicates that the determination could not be made on account of there being too little of the solution. After weighing the saturated solution in those cases where two layers were present it was transferred to a distilling bulb, diluted with several times its volume of water, the alcohol distilled and its amount determined by the pycnometer method. The aqueous solution remaining after the distillation was transferred to a weighing bottle, evaporated to dryness, and the residue weighed after being dried to constant weight at 150°. The composition of the residues was ascertained in most cases by a determination of the potassium in the manner mentioned for the original material used for the solubility experiments. In those cases where the residues came from the strong alcoholic solutions or upper layers, that is, with relatively very little of the salt present, the correction for impurity on the basis of the potassium determinations was very great.

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[a] Am. Chem. Jour., **14**, 380, 1892.

TABLE No. XIX.—*Solubility of potassium citrate in aqueous alcohol solutions at 25° C.*

No.	Per cent by weight of C_2H_5OH in original solvent.	Specific gravity of saturated solution at 25°.	Per cent by weight of C_2H_5OH in saturated solution.	$(CH_2)_2COH(COOK)_3 + H_2O$ per 100 grams saturated solution.
				Grams.
1	0.0	1.5180	0.0	64.50
2	8.9	[a]	....	......
		[b] 1.4920	0.0	60.00
3	32.0	[a]	....	0.2
		[b] 1.4930	0.0	61.60
4	51.0	[a]	65.1	0.38
		[b]	....	62.50
5	70.2	[a] 0.8366	81.0	0.10
		[b]	....	62.30
6	81.4	0.8356	81.4	0.038
7	91.6	0.8139	91.6	0.016
8	99.9	0.7896	99.9	0.014

[a] Upper layer. [b] Lower layer.

One hundred grams H_2O dissolve 181.8 grams $(CH_2)_2COH(COOK)_3 + H_2O$ or 1 gram of the salt requires 0.55 grams H_2O for solution at 25°.

The results which were obtained are given in Table No. XIX and show that two layers are formed in alcoholic solutions of less than 80 weight per cent alcohol. An interesting point is that in the lower layers of Nos. 2 and 3 although no alcohol could be found, the amounts of citrate dissolved were considerably less than in tube No. 1 in which the solvent was water. In the case of the solutions of high alcoholic content the solubility, as might be expected, diminishes with increase of alcohol. In those cases, however, where the alcoholic solution was in contact with the aqueous layer this relation is apparently not so rigid, as will be seen by comparing the figure for tube No. 6 with that for the upper layer of tube No. 5 and also the case of the upper layer of tube No. 3, which can only be explained on the assumption that the alcoholic concentration is greater than in the upper layer of tube No. 4.

A comparison of the results here shown with those quoted by the U. S. Pharmacopœia shows that the present figure for water is considerably lower than that quoted, which is 1 part of salt in 0.5 parts of water at 25°, or 200 grams of salt per 100 grams of water. Of the other results found in the literature for the solubility in water that of Greenish and Smith[a] for 15.5° is given as 1 part in 0.65 parts of water, the d_{15} of the saturated solution being 1.519. If this result is calculated to the basis selected for stating the present results it is seen that their value becomes 162.9 grams $(CH_2)_2COH(COOK)_3+H_2O$ per 100 grams of H_2O. Another figure for the solubility of potassium citrate in water is given by Köhler,[a] who finds that 100 grams H_2O dissolves 169.7 grams anhydrous potassium citrate at 31.25°. This result calculated to the hydrated salt becomes 199.9 grams $(CH_2)_2COH(COOK)_3+H_2O$ per 100 grams of H_2O at 31.25°. Considering, therefore, the three available determinations, we have for

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[a] Pharmaceutical Journal. (Lond.). June 22, 1901.

15.5°, 162.9 grams $(CH_2)_2COH(COOK)_3+H_2O$ per 100 grams H_2O
25.0°, 181.9 grams $(CH_2)_2COH(COOK)_3+H_2O$ per 100 grams H_2O
31.25°, 199.9 grams $(CH_2)_2COH(COOK)_3+H_2O$ per 100 grams H_2O

from which the following interpolated values are obtained:

t°	$(CH_2)_2COH(COOK)_3+H_2O$ per 100 grams.	
	Saturated solution.	Water.
	Grams.	*Grams.*
15	61.8	162.0
20	63.2	172.0
25	64.5	182.0
30	66.0	194.0

As regards the solubility of potassium citrate in alcoholic solutions, most of the reference books give only a qualitative statement that it is sparingly soluble. In Squire's Companion to the British Pharmacopœia, however, it is stated that 1 part of the salt dissolves in 9 parts of 60 per cent alcohol "but if more of the salt is added the alcohol separates from the watery solution," thus recognizing the power of potassium citrate to salt alcohol out of its aqueous solution.

In order to obtain exact figures upon this particular action of potassium citrate a series of determinations was made with alcoholic solutions of known concentrations and weighed amounts of the recrystallized potassium citrate. The method employed was as follows: To a measured volume of the alcoholic solution contained in a glass-stoppered bottle there was added enough potassium citrate from a weighing bottle to cause a distinct cloudiness upon shaking. The amount of the citrate which had been added was determined by noting the difference in weight of the weighing bottle after the addition of the citrate. The temperature of the cloudy solution was then brought to as near 25° as possible and successive amounts of the same alcoholic solvent added from a burette until the cloudiness just disappeared upon shaking thoroughly. The specific gravity of the clear solution was then determined by the pycnometer method. The weight of the potassium citrate divided by the total volume of the alcohol employed gave the grams of citrate per unit volume of alcohol. The results which were obtained are given in Table No. XX and the curve in figure 3. They yielded a very smooth curve when plotted on cross-section paper and from this curve the interpolated values shown in the lower part of the table were obtained. Although these results indicate that the value quoted in Squire's Companion to the British Pharmacopœia is much too high, an exact comparison can not be made on account of the difference in temperature in the two cases. An experiment was therefore made at 15°, using alcohol of approximately 59 volume per cent strength. It was found that 100 cubic centimeters of this alcohol just remained clear upon the addition of 4.51 grams $(CH_2)_2COH(COOK)_3 + H_2O$, which corresponds to about 22 parts of alcohol per 1 of citrate instead of 9 parts per 1 of citrate as quoted by Squire's Companion.

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[a] Z. Ver. Zuckerind, **47**, 447, 1897.

TABLE No. XX.—*Showing the amounts of potassium citrate necessary to cause the separation of a second liquid phase in aqueous alcohol solutions of different concentrations at 25° C.*

EXPERIMENTAL DETERMINATIONS.

Aqueous alcoholic solvent.		Specific gravity of saturated solution at 25°.	$(CH_2)_2COH(COOK)_3 + H_2O$ per—	
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		100 c. c. solvent.	100 grams saturated solution.
			Grams.	*Grams.*
Dist. H_2O	0.0	1.5180	181.80	64.50
0.9854	8.9	1.3250	84.70	46.20
0.9545	32.0	1.0710	23.50	19.60
0.9359	41.7	0.9945	11.40	10.90
0.9164	51.0	0.9361	4.73	4.90
0.8718	70.2	0.8678	0.38	0.43

CALCULATED RESULTS.

The above values plotted on cross-section paper yielded a curve from which the following figures were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$(CH_2)_2COH(COOK)_3 + H_2O$ per—	
		100 c. c. solvent.	100 grams saturated solution.
		Grams.	*Grams.*
0.0	1.518	181.80	64.50
5.0	1.400	111.00	52.50
10.0	1.310	80.00	45.50
20.0	1.177	47.00	31.50
30.0	1.085	26.50	21.50
40.0	1.005	13.20	12.40
50.0	0.943	5.50	5.60
60.0	0.900	1.50	1.60
70.0	0.868	0.40	0.40
80.0	0.838	0.04	0.04

Solubility of sodium citrate in aqueous alcohol solutions.—A sample of this salt purchased for solubility determinations was analyzed by incineration and titrating, as usual, but the results when calculated to $(CH_2)_2COH(COONa)_3+5\frac{1}{2}H_2O$ corresponded to 119.6 per cent. It was therefore evident that considerable dehydration had taken place, and in order to obtain a sample containing the desired amount of water of crystallization it would be necessary to recrystallize under proper conditions. According to a statement in Beilstein the salt crystallizes above 60° with $2H_2O$; therefore the following precautions were taken: About 100 to 150 grams of the salt were dissolved in just enough water to yield an almost saturated solution at 50°. The beaker was then placed outside the window, where the temperature remained below about 5° for several days. The mother liquor was drained off and the crystals powdered, placed in a bottle, and allowed to stand in a vacuum desiccator containing a few lumps of $CaCl_2$ for about a week. The analysis showed a composition of 98.7 per cent $(CH_2)_2COH)COONa)_3+5\frac{1}{2}H_2O$, which is considerably above the pharmacopœial requirement of 97 per cent. A calculation to salt of $6H_2O$ corresponded to 102.0 per cent, showing that the impurity was most probably adhering moisture. The mother liquor from the above crystallization was boiled until a scum appeared, cooled slightly, and the crystals which separated filtered on a Buchner funnel, dried in a desiccator for about a week, and analyzed. The results corresponded to 95.4 per cent $(CH_2)_2COH(COONa)_3+2H_2O$. This product was then dried at 150° for about five hours and gave results upon analysis corresponding to 98.5 per cent anhydrous salt. The acidity of the sample corresponded to 0.34 per cent anhydrous citric acid.

The solubility determinations were made with both the salt containing $5\frac{1}{2}$ H_2O and the anhydrous material. A smooth curve was obtained, and the only difference in the two sets of results was that the determinations made with the anhydrous salt were slightly below those made with the hydrated salt, due, no doubt, to the tendency of the dehydrated salt to take water from the solvent, and thereby increase the alcoholic concentration with resulting apparent lowering of the solubility in the particular solvent. Two series of determinations were made with the crystallized salt, the periods of shaking being two and three days, respectively. The weighed saturated solutions were transferred to weighing bottles, evaporated to dryness, and the residues dried to constant weight at 150° to 180°. Certain of the residues were analyzed by determination of the sodium in order to check the completeness of the dehydration. The weights of the hydrated salt were calculated from the anhydrous residues obtained. The results are given in Table No. XXI, and the curve shown in figure 3.

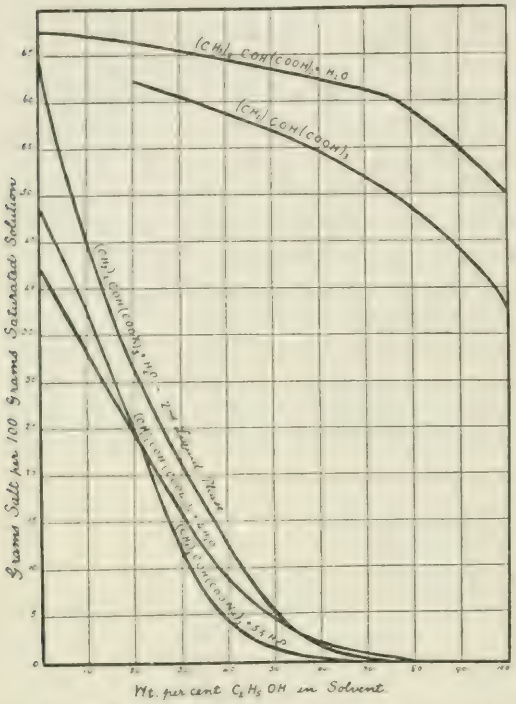

FIG. 3.—Curves showing the solubilities of citric acid and the citrates in aqueous alcohol solutions at 25°.

The present figure for the solubility of sodium citrate in water agrees fairly well with the value quoted by the U. S. Pharmacopœia, which is 1 part in 1.1 parts H_2O, or 90.9 grams salt per 100 grams H_2O. The statement regarding the solubility in alcohol should, however, be modified since the salt is evidently practically insoluble in pharmacopœial alcohol instead of slightly soluble, as reported. There are apparently no other results upon the solubility of this salt to be found in the literature.

TABLE NO. XXI.—*Solubility of sodium citrate in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$(CH_2)_2COH(COONa)_3 5\frac{1}{2}H_2O$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.2760	48.10
0.9856	8.9	....	38.90
0.9752	17.0	1.1290	29.30
0.9628	26.4	1.0370	15.70
0.9359	41.7	0.9473	3.70
0.9164	51.0	0.9166	1.35
0.8718	70.2	0.8654	0.09
0.7941	99.9	0.7894	....

TABLE XXI.—*Solubility of sodium citrate in aqueous alcohol solutions at 25°*—Cont'd.

CALCULATED RESULTS.

The above figures plotted on cross-section paper gave a curve from which the following results were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$(CH_2)_2COH(COONa)_3+5\frac{1}{2}H_2O$ per 100 grams.		Solvent to dissolve 1 gram $(CH_2)_2COH(COONa)_3+5\frac{1}{2}H_2O$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0	1.276	48.1	92.7	1.08
10	1.190	37.4	59.7	1.67
20	1.100	25.0	33.3	3.00
30	1.006	11.8	13.4	7.57
40	0.953	4.5	4.7	21.22
50	0.918	1.4	1.4	70.40
60	0.892	0.3	0.3	332.40
100	0.789	....	....	....

GALLIC ACID.

Solubility of gallic acid in aqueous alcohol solutions at 25°.—There is no satisfactory method by which gallic acid can be quantitatively determined, and therefore an analysis of a given sample can not be made. Certain qualitative tests are prescribed by the Pharmacopœia but probably would not indicate the presence of as small amounts of impurities as would be considered undesirable in a sample to be used for accurate solubility determinations. In order, therefore, to obtain material which might be considered of satisfactory quality for the solubility determinations desired for the present bulletin, a sample of apparent good quality was subjected to recrystallization from hot water, dried in a vacuum desiccator, and the water of crystallization determined by loss of weight on drying at 100°. The result obtained was 9.57 per cent, instead of the theoretical 9.58 per cent, therefore indicating a high degree of purity.

The solubility determinations were made with the recrystallized product in the usual manner, the time allowed for solution being two days. The weighed saturated solutions were evaporated to dryness in weighing bottles and the residues rendered anhydrous by drying at 100° and subsequently at 125° to 140° without further loss of weight. The weight of dissolved hydrated gallic acid was calculated from the anhydrous residues. The values yielded a perfectly smooth curve from which the interpolated results were obtained. The figures are given in Table No. XXII, and the curve shown in figure 4.

It will be noted that the values for water and alcohol of pharmacopœial strength are in fair agreement with those quoted by the U. S.

Pharmacopœia which are 1 part of acid in 83 to 86 parts of water and in 4.14 parts of alcohol.

TABLE NO. XXII. *Solubility of gallic acid in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_6H_2(OH)_3COOH+H_2O$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.002	1.15
0.9752	17.0	0.985	3.31
0.9359	41.7	0.974	11.16
0.8718	70.2	0.946	18.01
0.8234	89.6	0.919	21.17
0.7941	99.9	0.902	22.14

CALCULATED RESULTS.

The above determinations plotted on cross-section paper yielded a curve from which the following results were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$C_6H_2(OH)_3COOH+H_2O$ per 100 grams.		Solvent to dissolve 1 gram of $C_6H_2(OH)_3COOH+H_2O$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.002	1.15	1.16	86.00
10.0	0.992	2.0	2.04	49.00
20.0	0.983	4.2	4.38	22.80
30.0	0.977	7.5	8.11	12.30
40.0	0.972	10.6	11.86	8.60
50.0	0.965	13.4	15.47	6.46
60.0	0.957	16.0	19.04	5.25
70.0	0.946	18.0	21.96	4.55
80.0	0.933	19.9	24.85	4.02
90.0	0.919	21.2	26.90	3.72
[a] 92.3	0.915	21.4	27.23	3.67
95.0	0.911	21.6	27.55	3.63
100.0	0.902	22.2	28.54	3.50

[a] U. S. Pharmacopœia strength.

The solubility of gallic acid in several solvents has been determined by Rosenheim and Schidrowitz[a] and their results published in a paper with the title "The Optical Activity of Gallotannic Acid." The form in which the results are given is misleading since it is impossible to know whether the figures represent grams of hydrated or anhydrous acid dissolved in the given weight of solvent or of the saturated solution. If we assume, however, that the basis intended is the grams per 100 grams of solvent, their results are, respectively, for water 0.76 gram gallic acid per 100 grams at 12.5°, for 90 per cent alcohol (weight or volume?) 18.9 grams per 100 grams alcohol, and for absolute alcohol 22.2 grams acid per 100 grams alcohol at 15°.

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[a] J. Chem. Soc., (Lond.), **73**, 882, 1898.

A figure which is very much at variance with the present results and all others reported in the literature is quoted by Squire's Companion to the British Pharmacopœia as 1 part of gallic acid in 8 parts of 90 per cent alcohol. This is evidently an error, possibly typographical.

Solubility of gallic acid in several organic solvents.—The same recrystallized material was used for these determinations and the solvents which have already been described. The saturated solutions were transferred to weighing bottles and evaporated to dryness and the residues dried to constant weight at a little over 100°. It will be seen that with the exception of acetone and alcohol none of the solvents dissolved very large amounts of the acid. Some of the present results differ considerably from those of Rosenheim and Schidrowitz,[a] made at 15°. These authors do not describe the method by which their determinations were made and a correct opinion of their accuracy can not be formed.

The comparative results are as follows:

Solvent.	R. and S., 15°.	Present results, 25°.
Acetone	29.4	26.0
Alcohol (abs.)	22.2	22.1
Ethyl acetate	8.4	3.6
Benzene		0.02
Chloroform		
Ether (abs.)	2.5	1.37

Table No. XXIII.—*Solubility of gallic acid in organic solvents at 25°.*

Solvent.	Specific gravity of solvent.	d_{25} of saturated solution.	$C_6H_2(OH)_3COOH+H_2O$ per 100.			Solvent to dissolve 1 gram $C_6H_2(OH)_3COOH+H_2O$.
			Grams saturated solution.	Grams solvent.	C. c. Solvent.	
			Grams.	*Grams.*	*Grams.*	*Grams.*
Acetone	$d_{15}=0.797$	0.941	25.990	35.120	27.99	2.85
Amyl alcohol (iso)	$d_{20}=0.817$	0.834	5.390	5.700	4.65	17.55
Amylacetate	$d_{20}=0.875$	0.878	2.720	2.800	2.45	35.77
Benzene	$d_{25}=0.873$	0.875	0.022	0.022	0.02	4544.00
Carbon bisulphide	$d_{25}=1.258$	1.262	0.042	0.042	0.05	2381.00
Ether (abs.)	$d_{22}=0.711$	0.718	1.370	1.390	0.99	72.00
Ethyl acetate	$d_{25}=0.892$	0.911	3.610	3.750	3.34	26.70

The amounts of gallic acid dissolved by the following solvents were too small for estimation: Carbon tetrachloride, chloroform, and toluene.

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[a] Loc. cit.

LACTIC ACID.

Lactic acid in its usual form is a very hygroscopic syrupy liquid composed, according to the requirements of the Pharmacopœia, of not less than 75 per cent by weight of absolute lactic acid ($CH_3CHOH{\cdot}COOH$) and about 25 per cent of water. It is freely miscible with water, alcohol, and ether. A sample analyzed by titration with normal alkali using phenolphthaleine as indicator was found to contain 72.8 per cent $CH_3CHOH{\cdot}COOH$. It has been shown by Krafft and Dÿes[a] that the ordinary commercial lactic acid can be purified by distillation under diminished pressure (about 1 mm.) and the distillate so obtained is of 98.99 per cent purity and solidifies to a crystalline mass when cooled in an ice mixture. The crystals are very hygroscopic and the melting point is approximately 18°. A product of this character is no doubt the kind which should be used for quantitative solubility determinations but the results would probably be of little interest from the standpoint of the pharmacopœia at present. On this account it was not considered necessary to undertake other than approximate determinations with the pharmacopœial lactic acid.

A few experiments were therefore made with the lactic acid of 72.8 per cent referred to above and a number of the organic solvents used for the determinations, with other acids as described in the preceding pages of this bulletin. The plan of the experiments was simply to add the lactic acid from a burette to the measured quantities of organic solvent contained in a glass stoppered bottle and note the point where opalescence occurred on shaking. It was found that 20 cubic centimeter portions of benzene, carbon tetrachloride, chloroform, carbon bisulphide, nitro benzene, and toluene each required less than 0.1 cubic centimeter of the lactic acid to give the opalescence on shaking. The solubility in these solvents is therefore not greater than one part in 200, and is probably even very much less than this figure. In the cases of amyl alcohol, amyl acetate, acetone, ethyl acetate, and ether, the addition of equal volumes of the lactic acid did not produce opalescence. On adding water, however, to the mixture a second layer separated in all cases except with acetone. In the cases of amyl alcohol and acetate the volumes of the upper or organic solvent layer appeared about equal to the volume originally used; in the case of ethyl acetate and ether the upper layers appeared considerably smaller than the volume of each used in the beginning.

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[a] Ber. 28, 2589, 1895.

OLEIC ACID.

Solubility of oleic acid in aqueous alcohol solutions.—The sample used for the following experiments was pale amber colored and had specific gravity of 0.8969 at 20° and 0.8935 at 25°. A quantitative determination made by dissolving 10.1089 grams in enough alcohol to make 100 cubic centimeter of solution and titrating 10 cubic centimeter portions of this with standard 0.1 normal alcoholic potassium hydroxide, phenolphthaleine being used as indicator, gave results indicating a purity of 99.5 per cent $C_8H_{17}CH:CH(CH_2)_7COOH$. The preliminary solubility determinations were made by titrating aqueous alcohol solutions of increasing alcoholic content with the above oleic acid. In the case of all solutions below 50 weight per cent alcohol, the first drop of acid caused an opalescence on shaking, therefore indicating a solubility of less than about 0.05 grams acid per 100 cubic centimeters of alcohol. With solutions containing more than 50 per cent alcohol the following results were obtained:

Per cent by weight of C_2H_5OH.	Oleic acid per 100 c. c. alcohol to produce cloudiness.	Remarks.
	C. c.	
51.0	0.08–0.2	Cloudiness increased with continued addition of acid.
58.2	0.2 –0.4	Do.
65.5	0.3 –0.6	Cloudiness increased until about 5.5 c. c. had been added and then solution cleared.
70.2	0.6 –1.0	Cloudiness disappeared when about 4.5 c. c. acid had been added.
81.4	∞	No cloudiness appeared at all.

From these results it appears that below about 50 weight per cent alcohol the oleic acid is practically insoluble, between about 50 and 70 weight per cent the solubility apparently increases very gradually and then at about 75 per cent alcohol it goes up very abruptly to probable complete miscibility.

It should be mentioned that the point of the appearance of opalescence caused by adding the oleic acid to the alcohol is very uncertain. The cloudiness is at first very faint and increases gradually, so that no definite end point can be selected. Since it was observed that by continued addition of the oleic acid to the alcoholic solution of 70.2 weight per cent a point was reached at which the amount of opalescence began to diminish and finally disappeared entirely, it was decided to attempt to find the exact end points by titrating backwards; that is, by dissolving an excess of the oleic acid in the aqueous alcohol and then adding water until opalescence reappeared. Working in this way it was found that a very sharp end point could be obtained, one drop of water in excess being sufficient to cause the perfectly clear solution to become distinctly cloudy on shaking. The values obtained by these two methods were widely different, however, evidently corresponding to two sets of conditions. In the one case the end point was marked only by an increasing cloudiness, while in the other the perfectly clear solution was transformed by one drop of the added solvent to an opalescent mixture, which after a few minutes' standing separated into two liquid layers, the volume of neither of which corresponded to the one drop of water which caused the separation. A series of experiments made according to the last-mentioned method of determination was made as follows:

Amounts of oleic acid varying between 2 and 25 cubic centimeters were added to definite volumes of 70.2 weight per cent alcohol (65.5 weight per cent was used, however, in one case and 81.4 per cent in another), and the mixtures were then titrated to appearance of opalescence with water, care being taken to bring the temperature just to 25° before reaching the end point. One determination (No. 7) was made by continuing the titration with the oleic acid until opalescence appeared, thus making the determination direct and not by back titration. The result obtained in this case was in satisfactory agreement with the others, showing that it is immaterial whether the end point with these concentrations of oleic acid be approached from one or the other direction. The quantities used in the titration were as follows:

Determination No.	C_2H_5OH in solvent, per cent by weight.	Amount of alcoholic solvent.	Oleic acid added.	H_2O for back titration.
		c. c.	c. c.	c. c.
1	70.2	25.0	2.0	3.90
2	70.2	25.0	4.0	3.70
3	[a] 65.5	26.5	5.0	1.75
4	70.2	25.0	8.0	2.75
5	70.2	25.0	12.5	1.55
6	70.2	35.0	25.0	1.00
7	70.2	25.0	23.2	....
8	81.5	1.5	10.0	0.05

[a] Made from 70.2 weight per cent alcohol by adding 1.55 c. c. H_2O to 25 c. c. of the alcohol.

From the above results it is possible to calculate the amounts of the three components, water, alcohol, and oleic acid which are present in each case and from these values the percentage of alcohol in each solvent (water + alcohol) at the end of the titration and also the weight of oleic ocid present per 100 grams of the saturated solution. This has been done and the results which were obtained are given in the accompanying Table No. XXIV.

TABLE No. XXIV.—*Showing the amounts of oleic acid necessary to cause the separation of a second liquid phase in aqueous alcohol solutions of different concentrations at 25°.*

CALCULATED VALUES.

[From experimental determinations shown on p. 52.]

Determination No.	Composition of saturated solution.			Calculated per cent by weight of C_2H_5OH finally in solvent.	Oleic acid per 100 c. c. of alcoholic solvent.	Oleic acid per 100 grams saturated solution.
	C_2H_5OH.	H_2O.	Oleic acid.			
	Grams.	*Grams.*	*Grams.*		*c. c.*	*Grams.*
1	15.300	10.400	1.794	59.50	6.92	6.53
2	15.300	10.200	3.588	60.00	13.93	12.34
3	15.300	9.800	4.485	60.95	17.70	15.16
4	15.300	9.250	7.175	62.30	28.83	22.60
5	15.300	8.050	11.210	65.50	47.09	32.40
6	24.420	10.100	22.420	67.95	69.44	41.57
7	15.300	6.500	20.810	70.20	92.81	51.57
8	1.195	0.321	8.969	78.80	645.20	85.10

INTERPOLATED RESULTS.

These values plotted on cross-section paper give practically straight lines, from which the following results were obtained.

Per cent by weight of C_2H_5OH in solvent.	Oleic acid per 100 c. c. solvent.	Oleic acid per 100 grams saturated solution.
	c. c.	*Grams.*
57.0	....	0.0
58.5	0.0	5.0
60.0	11.0	12.3
62.5	30.0	20.0
65.0	49.0	30.5
67.5	69.0	40.0
70.0	91.0	50.0
75.5	....	68.5
80.0	....	88.0

From these results it is seen that the amount of oleic acid which will remain in a homogeneous mixture with alcohol and water increases very rapidly with relatively slight increases of alcoholic content beginning in solutions containing about 57 weight per cent alcohol. That part of the curve (or rather straight line, see figure 4) between 0 and about 5.0 grams of oleic acid per 100 grams of the saturated solution can not be determined directly since this quantity of oleic acid is not sufficient to yield a clear solution before the back titration with the water. In regard to the upper limit of the amount of oleic acid in the alcoholic solutions of higher concentrations it appears that the point of complete miscibility is reached at about 80 to 85 weight per cent alcohol, and therefore no second liquid layer can be made to separate in alcohol-water mixtures containing more than this per cent of alcohol.

As has already been mentioned, the opalescent solutions which are produced by the addition of the last drop of water in the back titration, separate into two layers when allowed to stand. These solutions which resulted in the titration numbers 1 to 6 (see p. 52), were transferred to graduated cylinders and allowed to stand 24 hours and the volumes of the upper and lower layers then read. The perfectly clear layers of which the upper was yellowish and the lower nearly white or pale straw colored in each case were then analyzed by withdrawing enough to fill a pycnometer, weighing and titrating the dissolved oleic acid with alcoholic sodium hydroxide solution. An unsuccessful attempt was made in one case to determine the alcohol present by neutralizing the oleic acid with aqueous alkali and distilling. The excessive foaming, however, prevented a successful distillation of the alcohol and the procedure was abandoned. The results which were obtained are as follows. The figures in parentheses indicate estimated values. Some loss occurred in transferring the solutions from the titration flasks to the graduated cylinders, and therefore the sum of the volumes of the upper and lower layers are less than that of the solutions mixed as given for the titration results on page 52. This loss also accounts for the differences in the amounts of oleic acid found and those originally added.

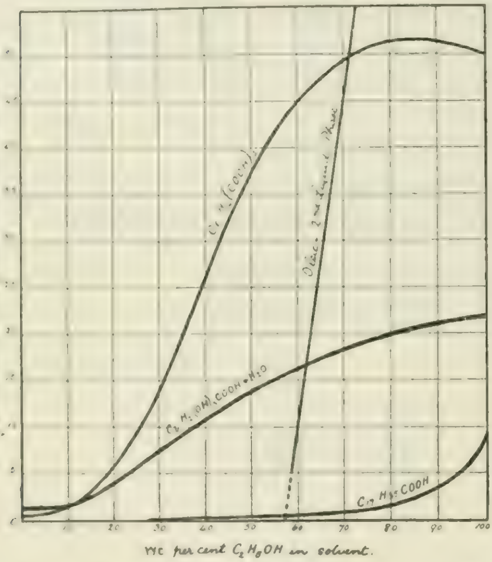

Fig. 4. Curves showing the solubilities of camphoric, gallic, oleic, and stearic acids in aqueous alcohol solutions at 25°.

Determination No.	Lower layer.				Upper layer.			
	Volume of lower layer.	Specific gravity.	Oleic acid per 100 grams solution.	Oleic acid in lower layer.	Volume of upper layer.	Specific gravity.	Oleic acid per 100 grams solution.	Oleic acid in upper layer.
	c. c.		*Grams.*	*c. c.*	*c. c.*		*Grams.*	*c. c.*
1	29.0	0.893	5.123	1.48	1.0	....	....	(0.35)
2	26.0	[illegible]	7.347	1.89	6.0	(0.875)	33.83	1.98
3	22.7	0.891	8.572	1.93	9.3	(0.875)	31.39	2.78
4	16.0	0.893	6.139	0.98	19.0	0.876	35.54	6.59
5	6.0	(0.890)	6.259	0.37	33.2	0.878	36.61	11.87
6	4.5			(0.28)	55.5	0.877	44.59	24.14

From these results it appears that the composition of each of the upper layers and of each of the lower layers is nearly the same in all cases. The percentage of oleic acid in the upper averages about 36, and in the lower about 7.

The statements in the literature in regard to the solubility of oleic acid are mostly to the effect that it is insoluble in water and soluble in alcohol. The present results verify this statement as far as the water is concerned, but show that with alcohol of less than 80 weight per cent the insolubility may vary over a very considerable range, depending upon slight changes in the alcoholic concentration.

PHENOLSULPHONATES.

Solubility of sodium phenolsulphonate in aqueous alcohol solutions.—In igniting this compound, as mentioned in the Pharmacopœia, the residue at first contains unburned carbon which on heating to a higher temperature reduces a part at least of the sulphate to sulphide, thus not yielding a white residue of sodium sulphate amounting to 30.6 per cent of the original weight as stated. It was found that a fairly good determination could be made by first drying the weighed samples in the air bath at 120° to 140°, and after having cooled and weighed the residue to determine the loss of water of crystallization, igniting it very carefully so that no flaming occurs. The charred residue is then treated with a few drops of concentrated nitric acid and heated to redness. This procedure is repeated until a perfectly white residue is obtained. Determinations made in this way upon the sample to be used for the solubility experiments showed the presence of 15.44 per cent of water of crystallization instead of the theoretical 15.52 per cent, and gave sodium sulphate corresponding to 98.9 per cent $C_6H_4(OH)SO_3Na+2H_2O$.

One series of solubility determinations was made, the time of shaking being two days. The weighed saturated solutions were transferred to weighing bottles and evaporated to dryness, the residues were dried to constant weight at 140°, and in addition some were analyzed as mentioned above for the sodium, and in this way the completeness of the drying was verified. The results which were obtained are given in Table No. XXV, and the curve from them in figure 5. The figures for water and pharmacopœial alcohol are in fair accord with those reported by the pharmacopœia which are respectively 1 part in 4.8 water and 1 part in 130 parts alcohol. A determination made by Greenish and Smith[a] at 15° gave 1 part of $C_6H_4(OH)SO_3Na+2H_2O$ per 5.48 parts of water, the specific gravity of the saturated solution being 1.0675.

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[a] Pharm. Jour., (Lond.) June 22, 1901.

TABLE No. XXV.—*Solubility of Sodium Phenolsulphonate (para) in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_6H_4(OH)SO_3Na+2H_2O$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist H_2O	0.0	1.079	19.38
0.9856	8.9	1.057	17.46
0.9752	17.0	1.038	15.98
0.9628	26.4	1.016	14.47
0.9164	51.0	0.952	[a] 10.23
0.8718	70.2	0.886	5.03
0.8234	89.6	0.821	1.16
0.7941	99.9	....	[b] 1.49

[a] This result unaccountably about 0.7 per cent high.
[b] Solid phase became opaque.

CALCULATED RESULTS.

The above figures plotted on cross section paper gave a curve from which the following results were obtained.

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$C_6H_4(OH)SO_3Na+2H_2O$ per 100 grams.		Solvent to dissolve 1 gram $C_6H_4(OH)SO_3Na+2H_2O$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.079	19.4	24.1	4.16
10.0	1.054	17.4	21.2	4.71
20.0	1.030	15.5	18.3	5.45
30.0	1.004	13.6	15.7	6.35
40.0	0.977	11.7	13.3	7.55
50.0	0.950	9.7	10.7	9.31
60.0	0.919	7.5	8.1	12.30
70.0	0.886	5.1	5.4	18.60
80.0	0.852	2.9	3.0	33.50
90.0	0.820	1.1	1.1	89.90
92.3	0.815	0.9	0.9	110.10
95.0	0.810	0.8	0.8	124.00
100.0	0.800	1.5	1.5	65.70

Solubility of zinc phenolsulphonate in aqueous alcohol solutions.—The sample used for the following solubility experiments was analyzed by determining the water of crystalization and by precipitating the zinc as carbonate from the hot solution by means of sodium carbonate, washing, igniting, and weighing as zinc oxide. The results were 25.85 per cent water of crystallization instead of the theoretical 25.93 per cent, and the zinc oxide corresponded to 100.7 per cent $Zn(C_6H_4(OH)SO_3)_2+8H_2O$. One series of determinations was made, the time of shaking being two days for some of the tubes and four for others. The weighed saturated solutions were evaporated in weighing bottles and the residues dried to constant weight at 130 to 140°.

The weights of anhydrous salt were calculated to the hydrated compound and these values converted to the quantities per 100 grams of the saturated solutions. The results are given in Table No. XXVI, and the curve in figure 5.

TABLE NO. XXVI—*Solubility of zinc phenolsulphonate (para) in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$(C_6H_4(OH)SO_3)_2Zn+8H_2O$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist H_2O	0.0	1.185	39.81
0.9856	8.9	1.174	40.11
0.9752	17.0	1.165	40.53
0.9628	26.4	1.155	41.24
0.9164	51.0	1.124	42.12
0.8718	70.2	1.080	40.85
0.8234	89.6	1.047	41.34
0.7941	99.9	1.075	48.77

CALCULATED RESULTS.

The above figures plotted on cross section paper gave a curve from which the following values were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$(C_6H_4(OH)SO_3)_2Zn+8H_2O$ per 100 grams.		Solvent to dissolve 1 gram $(C_6H_4(OH)SO_3)_2Zn+8H_2O$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.185	39.8	66.1	1.512
20.0	1.161	40.7	68.6	1.457
40.0	1.139	42.1	72.7	1.375
60.0	1.106	41.6	71.2	1.404
80.0	1.057	40.7	68.6	1.457
90.0	1.047	41.4	70.6	1.416
92.3	1.048	41.9	72.1	1.387
95.0	1.052	42.9	75.1	1.330
100.0	1.075	48.8	95.3	1.049

Maximum at 47 weight per cent C_2H_5OH, 42.2 grams $(C_6H_4(OH)SO_3)_2Zn+8H_2O$ per 100 grams saturated solution.

Minimum at 78 weight per cent C_2H_5OH, 40.7 grams $(C_6H_4(OH)SO_3)_2Zn+8H_2O$ per 100 grams saturated solution.

The figures quoted by the U. S. Pharmacopœia are somewhat below the present results. Some results by Greenish and Smith[a] and Squire[b] for 15° C. are even below those reported by the U. S. Pharmacopœia.

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[a] Pharm. Jour., (Lond.), i, 552, 1902; ibid., ii, 947, 1903.

[b] Companion to the British Pharmacopœia, 18th Ed.

The solubility curve for this salt is very striking in that it shows both a maximum and minimum point.

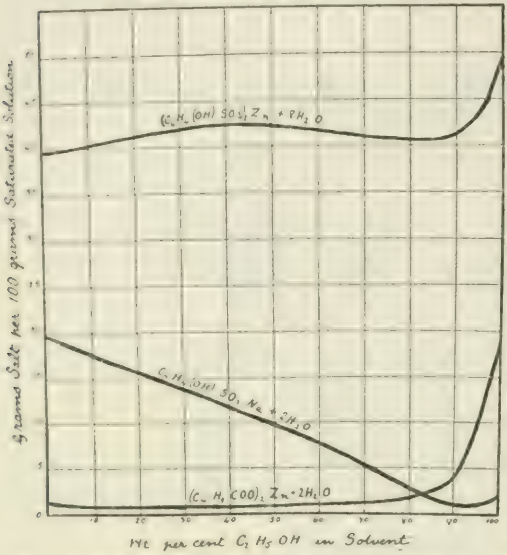

Fig. 5. Curves showing the solubilities of sodium and zinc phenolsulphonate and of zinc valerate in aqueous alcohol solutions at 25°.

SALICYLIC ACID AND THE SALICYLATES.

Solubility of salicylic acid in aqueous alcohol solutions at 25°.—The samples of salicylic acid used for the solubility determinations were analyzed by dissolving weighed samples in neutralized alcohol and titrating with standard alkali using phenolphthaleine as indicator. The results indicated a purity in all cases of at least 99.8 per cent. The melting point was found to be 158.2° to 159° (Cor.)[a]. One series of determinations was made, the time allowed for saturation being three days. The weighed saturated solutions were transferred to measuring flasks, diluted to the mark with neutralized alcohol and aliquot portions titrated with standard alkali. The results are given in Table No. XXVII and the curve shown in figure 6.

The curve rises at first very slowly and then at about 25 weight per cent alcohol goes up very rapidly to the solvent of 100 per cent alcoholic strength in which the solubility is 33.2 grams salicylic acid per 100 grams of the saturated solution.

Table XXVII.—*Solubility of salicylic acid in aqueous ethyl alcohol solutions at 25°.*

EXPERIMENTAL RESULTS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_6H_4OHCOOH$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.00	1.001	0.22
0.9856	8.90	0.986	0.336
0.9545	32.00	0.957	2.68
0.9164	51.00	0.945	12.82
0.8718	70.20	0.941	24.01
0.8279	88.00	0.932	31.03
0.8048	96.30	0.923	32.45
0.7941	99.80	0.919	33.20

[a] In a previous report of these results upon salicylic acid (cf. Proc. Am. Electro-chem. Soc., Albany Meeting, 1908) the m. pt. was given as 156°, but this figure refers to the uncorrected value.

TABLE NO. XXVII.—*Solubility of salicylic acid in aqueous ethyl alcohol solutions at 25°*—Continued.

CALCULATED RESULTS.

The above experimental determinations plotted on cross-section paper gave a curve, from which the following figures were read or calculated:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$C_6H_4OHCOOH$ per 100 grams.		Solvent to dissolve 1 gram $C_6H_4OHCOOH$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.001	0.22	0.22	453.50
10.0	0.984	0.38	0.38	262.00
20.0	0.970	0.80	0.81	124.00
30.0	0.959	2.20	2.25	44.45
40.0	0.951	5.90	6.27	15.95
50.0	0.945	12.20	13.90	7.20
60.0	0.943	18.30	22.41	4.46
70.0	0.941	24.00	31.58	3.17
80.0	0.937	28.30	39.48	2.93
90.0	0.930	31.40	45.77	2.19
[a] 92.3	0.928	31.90	46.85	2.13
100.0	0.919	33.20	47.50	2.11

[a] U. S. Pharmacopœia strength.

The solubility values for salicylic acid in water to be found in pharmaceutical literature vary considerably. Thus it is given as 1 part in 308 by the U. S. Pharmacopœia and from 1 part in 444 to 1 part in 550 by other reference books. The present value agrees closely with the average of the determinations compiled from the chemical literature, which are as follows:[a]

Temperature °C.	0	10	20	25	30	40	60	80
Grams $C_6H_4OHCOOH$ per 100 c. c. solution	0.08	0.12	0.18	0.22	0.27	0.37	0.82	2.05

As for the solubility in alcohol, the values from the pharmaceutical reference books vary from 1 part in 2 to 1 part in 3.5 parts. The result for alcohol of U. S. Pharmacopœial strength, viz, 92.3 weight per cent, according to the present results for 25° is seen to be 1 part in 2.13 parts by weight.

Solubility of salicylic acid in organic solvents at 25°.—In these determinations the excess of acid was shaken with the solvent in each case for two days at 25° and the clear saturated solution weighed in a pycnometer, transferred to a glass-stoppered bottle, and titrated with standard alkali, using phenolphthaleine as indicator. The results are given in Table No. XXVIII. Only a few determinations are to be found in the literature with which to compare the present results, and for most of these the different temperature standards make an accurate comparison impossible. In the case of benzene the value obtained by Walker and Wood[a] is 0.78 grams, $C_6H_4(OH)COOH$ per 100 grams, C_6H_6 at 25°, instead of 0.86 grams per 100 grams of benzene as reported in the accompanying table. These same authors also give the following figures for the solubility in acetone and in ether. One hundred cubic centimeters of acetone solution at 23° contained 31.3 grams of the acid and 100 cubic centimeters of the ethereal solution at 17° contained 23.4 grams. The weight of salicylic acid per 100 cubic centimeters of saturated ethereal solution as calculated from the accompanying results at 25° is 27.68 grams. According to several of the pharmaceutical reference books, the solubility of salicylic acid in chloroform is given at 1.25 grams per 100 grams $CHCl_3$ at about 15°, which is, as would be expected, somewhat below the value 1.67 grams shown below. A value for amyl alcohol is given by Hager as 1 part in 3.5 parts, which is somewhat above the present figure of 1 part per 4.89 parts at 25°.

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[a] Cf. Seidell's Solubilities of Inorganic and Organic Substances (1907), p. 274. (D. Van Nostrand Co.)

TABLE NO. XXVIII.—*Solubility of salicylic acid in organic solvents at 25°.*

Solvent.	d of solvent.	d_{25} of saturated solution.	$C_6H_4OHCOOH$ dissolved per 100.			Solvent to dissolve 1 gram $C_6H_4OHCOOH$.
			Grams saturated solution.	Grams solvent.	cc. solvent.	
			Grams.	*Grams.*	*Grams.*	*Grams.*
Amyl alcohol (iso.) . . .	$d_{20}=0.817$	0.878	20.47	25.73	21.02	4.89
Amyl acetate .	$d_{[illegible]}=0.875$	0.917	16.67	20.00	17.50	5.00
Benzene . .	$d_{[illegible]}=0.873$	0.875	0.85	0.86	0.75	116.60
Carbon bisulphide.	$d_{25}=1.259$	1.259	0.23	0.23	0.29	434.00
Carbon tetrachloride. . . .	$d_{25}=1.587$	1.587	0.25	0.25	0.40	399.00
Chloroform.	$d_{23}=1.476$	1.477	1.64	1.67	2.46	60.00
Cumene	$d_{20}=0.863$	0.889	0.86	0.87	0.75	115.00
Ether (abs.).	$d_{22}=0.711$	0.857	32.29	47.68	33.90	2.10
Ligroin	$d_{25}=0.714$	0.714	0.13	0.13	0.09	768.00
Nitrobenzene	$d_{25}=1.205$	1.200	2.18	2.23	2.68	44.90
Spirits of turpentine. . .	$d_{20}=0.865$	0.854	2.22	2.27	1.96	44.10
Toluene.	$d_{15}=0.872$	0.863	0.84	0.85	0.74	118.00
Xylene.	$d_{25}=0.861$	0.860	0.90	0.91	0.78	110.00

Methods for the determination of salicylates.—In beginning the work upon the solubilities of the salicylates it appeared desirable to find a method for the accurate analysis of the various samples required and for determining the quantities of the several salts dissolved. The experiments were directed in the first place toward those methods by which the salicylic radicle could be estimated. Of these, two are described in the literature as the Freyer bromate method and the Messinger and Vortmann iodine method, respectively. Attempts were therefore made to apply each of these methods to the salicylates described in the following pages, but the results were uncertain, and it was found that with both methods the limits of the conditions within which the reactions proceed according to the accepted equations are so narrow that the results obtained under ordinary conditions were of uncertain reliability. The details of the experiments which led to this conclusion have been published elsewhere[a] and therefore need not be given here.

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[a] J. Chem. Soc., Lond., **73**, 620, 1898.

In regard to the methods for the determination of the inorganic constituent of the salts it will be noted that the procedures recommended by the pharmacopœia vary for each of the three different basic constituents present, viz, for the sodium, lithium, and strontium salts. In the first case the sample is ignited and the ash titrated with standard acid; in the second it is mixed with ammonium sulphate and ignited in order to convert the lithium to lithium sulphate; with the last, concentrated sulphuric acid is prescribed as the agent for converting the residue to strontium sulphate. As has been shown in the case of sodium benzoate (p. 28), so also with sodium salicylate has it been found necessary to extract the first ignited residue of the sample with water and ignite the unburned carbon, adding the solution of this second ash to the first in order to have all the sodium in available form for the titration with standard acid. With this precaution the pharmacopœial method for sodium salicylate is entirely satisfactory.

Some years ago a method for the determination of salicylates was proposed by Barthe,[b] according to which an excess of hydrochloric acid was added to the aqueous solution of the sample of salicylate and the mixture evaporated to dryness at not over 50°. The residue, consisting of free salicylic acid and the chloride of the base, was then titrated with standard alkali, using phenolphthaleine as indicator, and subsequently the chloride was titrated with standard silver nitrate solution. In view of the experiments of Fresenius and Grünhut,[c] who found that satisfactory results for salicylic acid could not be obtained by any method of liberating salicylic acid, shaking out with volatile solvents and evaporating at a very low temperature, it did appear advisable to attempt the determination of salicylic acid as suggested by Barthe, but it was believed that the determination of the base by titration of the chloride might yield good results. Experiments along this line were therefore made, including, however, a modification for removing as much as possible of the free salicylic acid before titrating the chloride. The following results were obtained upon a sample of sodium salicylate which contained 100.2 per cent $C_6H_4OHCOONa$ on the basis of the determination made by the pharmacopœial method modified as above mentioned. 50 grams were dissolved in water and diluted to 500 cubic centimeters. An excess of HCl was added to aliquot portions of this solution, which were then evaporated to dryness on the steam bath and either filtered and just neutralized with alkali before the titration with standard silver nitrate, or the residue (determination No. 2) was gently ignited to remove the free salicylic acid before the titration for chlorides.

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[a] Seidell, J. Am. Chem. Soc., **31**, 1168–77. 1909.

[b] Bull. soc. chim., (3) **11**, 517, 1894.

[c] Z. anal. Chem., **38**, 292, 1899.

Determination No.	Salicylate solution.	$C_6H_4OHCOONa$.	0.1 N $AgNO_3$ required.	Calc. $C_6H_4OHCOONa$.
	C. c.	*Grams.*	*C. c.*	*Per cent.*
1	25	0.250	15.6	99.9
2	50	0.500	31.3	100.2
3	75	0.750	47.0	100.3

These results show that in the case of sodium salicylate this method is as reliable as that of the pharmacopœia; that it will prove equally so in the case of the other salicylates is hardly to be doubted. If further experiments should confirm this expectation we would then have a single procedure for these salts instead of three as is now the case.

Solubility of ammonium salicylate in aqueous alcohol solutions.—In the case of this compound two series of determinations were made with two samples of the salt. In one case the solutions were allowed two days for reaching equilibrium and in the other something over a week. It was evident that the shorter time was ample; in fact, it seems probable that even ten hours would have been sufficient. The two samples of material were each analyzed as follows: A weighed quantity of the salt was transferred to a 200 cubic centimeter flask, dissolved in water and diluted to the mark. Aliquot portions of the solution containing 1 to 2 grams ammonium salicylate were placed in a Kjeldahl distilling flask with 25 cubic centimeters of normal alkali. The liberated ammonia was distilled into 25 cubic centimeters of normal acid, and the excess of acid in the receiver titrated back with standard alkali and the excess of alkali in the distillation flask titrated back with standard acid. The amount of alkali equivalent to the ammonia in both cases agreed fairly satisfactorily. From these readings the amount of ammonia and thus of the ammonium salicylate in the aliquot portion of the solution was easily calculated. According to this method the two samples gave the following results expressed in the percentage of $C_6H_4OH.COONH_4$ present. Sample (a), 96.8 per cent; sample (b), 98.7 per cent. Although one of these samples was below the United States Pharmacopœia requirement and the other above, the solubility determinations made with them gave a curve in which no irregularities could be traced definitely to either sample. It may be mentioned that of the results shown in Table No. XXIX, the second, third, and sixth were made with sample (a), and the others with sample (b). The impurity in the two samples was apparently principally moisture, and it might therefore be expected that in the solvents of higher alcoholic content the presence of this water would impair the results. That it did not do so to an appreciable extent is probably due to the lack of greater exactitude in the method by which the determinations were made.

TABLE NO. XXIX. –*Solubility of ammonium salicylate in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_6H_4OHCOONH_4$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
1.00	0.00	1.148	50.8
0.986	8.9	1.137	50.8
0.955	32.0	1.104	49.3
0.872	70.2	1.014	42.0
0.828	88.0	0.946	33.1
0.819	91.4	0.932	30.5
0.805	96.3	0.901	26.5
0.794	99.8	0.875	22.3

CALCULATED DETERMINATIONS.

These results plotted on cross-section paper gave a curve from which the following figures were read or calculated:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$C_6H_4OHCOONH_4$ per 100 grams.		Solvent to dissolve 1 gram C_6H_4-$OHCOONH_4$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.148	50.8	103.2	0.969
20.0	1.122	50.3	101.2	0.988
40.0	1.088	48.3	93.4	1.070
50.0	1.067	46.7	87.6	1.141
60.0	1.042	44.7	80.8	1.237
70.0	1.015	42.0	72.4	1.381
80.0	0.979	38.0	61.3	1.631
85.0	0.958	35.0	53.9	1.857
90.0	0.936	31.6	46.2	2.165
[a] 92.3	0.925	30.0	42.86	2.333
95.0	0.907	27.8	38.5	2.596
100.0	0.875	22.3	28.7	3.484

[a] U. S. Pharmacopœia strength.

Attention should be called here to the observation that on diluting to 200 cubic centimeters the weighed portions of each of the saturated solutions, except the one in water alone, considerable opalescence resulted. This opalescence increased with increasing alcohol content of the solvent. The cause of this separation of a portion of the constituents of the compound may possibly have been due to a certain amount of dissociation of the ammonium salicylate by the alcohol, yielding some salicylic acid which remained dissolved in the alcohol but separated upon the addition of water. In withdrawing the aliquot portions from each of the 200 cubic centimeters dilutions for the determination of ammonia, the solutions were well shaken and no attempt made to use only the clear portion. The ammonia determinations were made by distilling as described for the analyses of the sample of salt employed. A comparison of the standard alkali neutralized in the distillation flask and of the amount equivalent to the liberated ammonia showed no regular differences with increase of alcoholic content, indicating that even if a portion of the salicylic acid of the compound was set free by the alcohol, the ammonia simultaneously liberated was not lost in the subsequent treatment of the solution.

The curve (see fig. 6) plotted from the determined results is found to descend regularly with increase of the strength of the alcohol. It bows upward as a result of the fact that the increase in the solubility of the ammonium salicylate proceeds slowly at first, but with increasing concentration of alcohol in the solvent its decrease is much more rapid. This bowing of the curve from a straight line shows that the water and alcohol in the several solvents do not act independently in their solvent action upon the ammonium salicylate; that is, the amount dissolved by any mixture of water and alcohol is more than the amount dissolved by the quantity of water present plus the amount dissolved by the quantity of alcohol present.

A comparison of the results of the present determinations with those quoted in the U. S. Pharmacopœia shows that the latter are somewhat higher. According to the U. S. Pharmacopœia, 1 gram of ammonium salicylate is dissolved by 0.9 grams H_2O and by 2.3 grams of official alcohol at 25° or 100 grams of water dissolve 111 gram ammonium salicylate and 100 grams official alcohol 46 grams of the salt. The present results are 103.2 and 42.9 grams, respectively, for the amounts dissolved by 100 grams of each by the two pure solvents.

Solubility of bismuth subsalicylate in aqueous alcohol solutions.—Solubility determinations were made upon a sample of "Bismuth Salicylate, Merck—basic 64 per cent Bi_2O_3" by the same procedure followed for the determinations already described. The amounts dissolved were determined in the usual way by evaporation and drying in a vacuum desiccator, but the quantities of the residues were so small that the results are not entirely free from criticism. When, however, the values were plotted on cross-section paper, the average curve drawn through them was no doubt very close to the true results for the particular sample employed. The figures read from this curve are as follows:

Per cent by weight of C_2H_5OH in solvent.	$C_6H_4OH{\cdot}COO{\cdot}OBi$ per 100 grams saturated solution at 25°.
	Grams.
0.0	0.010
20.0	0.015
40.0	0.022
60.0	0.036
80.0	0.065
90.0	0.095
[a] 92.3	0.105
100.0	0.160

[a] U. S. Pharmacopœia strength.

The statement in most of the pharmaceutical reference books is that bismuth subsalicylate is almost insoluble in water. The above figures will serve only to show about how insoluble the salt is, for it is evident that different samples will give different results, depending upon their actual composition.

Solubility of lithium salicylate in aqueous alcohol solutions.—The sample used for these solubility determinations was analyzed by the method given in the U. S. Pharmacopœia. According to this method, the weighed portion of the material is intimately mixed with anhydrous ammonium sulphate and the mixture fused, the amount of lithium sulphate obtained being calculated to salicylate. The original sample as received from the manufacturer, as well as a recrystallized portion of the same, yielded practically identical results by this method as did also the residues which were obtained from the solubility determinations after drying to constant weight in a vacuum desiccator. The average of these determinations was 95.5 per cent of anhydrous lithium salicylate, or 101.5 per cent of $C_6H_4OHCOOLi + \frac{1}{2}H_2O$. The lithium sulphate residues that were obtained were powdered and mixed and analyzed by means of a determination of SO_4. The calculated Li_2SO_4 was found in this way to be approximately 1 per cent too high, showing that the lithium salicylate itself was probably contaminated with some other alkali. Assuming this to be the case, the calculated 101.5 per cent of $C_6H_4OHCOOLi + \frac{1}{2}H_2O$ instead of an even 100 per cent would be easily explained by assuming the presence of admixed sodium or potassium salicylate. Since the process of recrystallization of the original material from strong alcohol had not yielded a better product, other attempts to effect an improvement were not made. The accompanying solubility determinations are therefore open to the criticism that more or less impure material was used. It is probable, however, that the errors due to the possible impurities of the sample are comparatively small and the results as given in the accompanying table No. XXX are of sufficient reliability for all practical purposes.

Table No. XXX.—*Solubility of lithium salicylate in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	C_6H_4OHCOO-$Li+\frac{1}{2}H_2O$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.209	56.0
0.986	8.9	Not det.	55.9
0.954	32.4	1.159	54.2
0.915	51.6	1.120	52.0
0.869	71.5	1.080	49.2
0.815	92.6	1.021	45.4
0.804	96.6	1.018	45.6
0.794	99.9	1.027	48.2

CALCULATED DETERMINATIONS.

The above results plotted on cross section paper yielded a curve from which the following results were obtained:

Weight per cent of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$C_6H_4OHCOOLi+\frac{1}{2}H_2O$ per 100 grams.		Solvent to dissolve 1 gram of $C_6H_4OHCOOLi+\frac{1}{2}H_2O$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0	1.209	56.0	127.3	0.786
10	1.195	55.9	126.8	0.789
20	1.180	55.4	124.3	0.805
30	1.163	54.7	120.8	0.828
40	1.144	53.7	116.0	0.862
50	1.124	52.5	110.5	0.905
60	1.104	51.1	104.5	0.957
70	1.083	49.5	98.0	1.021
80	1.056	47.5	90.5	1.105
90	1.026	45.8	84.5	1.183
[a] 92.3	1.020	45.6	83.8	1.193
100	1.027	48.2	93.1	1.075

[a] U. S. Pharmacopœia strength.

The amounts of lithium salicylate dissolved were determined by evaporating the solvents and drying the residues in a vacuum desiccator containing concentrated sulphuric acid at room temperature. In the case of a duplicate determination of the solubility in water, however, the solvent was evaporated in a drying oven at about 60°, and in this case the residue which was obtained was found upon analysis to be almost anhydrous lithium salicylate. It differed considerably in physical appearance from the crystalline residues obtained in the other cases and therefore lends further evidence to the conclusion that the ordinary crystalline lithium salicylate contains one-half molecule of water of crystallization instead of being anhydrous, as described in the U. S. Pharmacopœia.

The solubility curve (see fig. 6) presents one unexpected peculiarity in that a minimum point is reached at a concentration of about 95 weight per cent alcohol.

Very few results are given in the literature with which the present values can be compared. The only statement in the U. S. Pharmacopœia is to the effect that lithium salicylate is very soluble in water and alcohol. Results from other sources vary from 1 part in 0.75 to 1 part in 1.0 part of water and from 1 part in 2.0 parts to 1 part in 1 part of alcohol. These figures are uncertain, however, since they may refer to the anhydrous salt, or, as is more likely, to a temperature different from that at which the present values were determined.

Solubility of methyl salicylate in aqueous alcohol solutions at 25°.— This compound, being a liquid at ordinary temperatures, does not require the usual procedure for determination of its solubility in alcohol solutions that has been adopted for the solid compounds. The titration method described under ethyl acetate (p. 13) may be used with satisfactory results. The method there described was modified, however, to some extent in the present case in order to insure a more accurate control of the temperature, since the change of solubility with temperature is very great with this compound. The details of the determinations are as follows: The measured volume of the aqueous alcoholic solvent of determined strength is brought to nearly 25° in a 100 cubic centimeters Erlenmeyer flask; the methyl salicylate is added slowly from a burette until a permanent clouding is observed. A thermometer is kept in the flask and the temperature noted during the addition of the salicylate. The temperature of the flask can be controlled by warming with the hands or cooling under the cold-water faucet. The last few drops of methyl salicylate must be added just as the temperature stands at 25°. The burette is read and the specific gravity of the saturated solution is immediately determined by the pycnometer method. For the determinations of the solubility made at various temperatures (Table No. XXXII) the solvent was cooled to the lowest temperature desired, viz, 15°, and the reading of the methyl salicylate taken just on appearance of opalescence at this point; the temperature was then allowed to rise to 20° and the addition of methyl salicylate continued until the end point at this temperature was observed, and so on with the 25° and 30° determinations.

The sample of methyl salicylate (artificial oil of wintergreen) was purified by distillation and the fraction obtained at 220° to 221° (cor.) used for the solubility determinations. The specific gravity of both the original and the distilled portion of the sample was found to be 1.182 at 25°.

TABLE NO. XXXI.—*Solubility of methyl salicylate in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_6H_4OHCOOCH_3$ per 100 c. c. aq. alcohol.	$C_6H_4OHCOOCH_3$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.			
			Cubic centimeters.	*Grams.*
Dist H_2O	0.0	1.000	(Ca.) 0.1	0.12
0.955	32.0	0.955	0.5	0.61
0.9164	51.0	0.924	5.3	6.40
0.8718	70.2	0.944	34.0	31.56
0.8441	81.4	1.090	270.0	79.09
0.8234	89.6	....	∞	....

CALCULATED RESULTS.

The above determinations plotted on cross-section paper yielded a curve from which the following results were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$C_6H_4OHCOOCH_3$ per 100 grams.		Solvent to dissolve 1 gram C_6H_4OH $COOCH_3$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0	1.000	0.12	0.12	832.00
30	0.958	0.60	0.61	166.00
40	0.940	2.30	2.35	42.50
50	0.925	6.20	6.61	15.10
55	0.922	10.00	11.10	9.00
60	0.923	18.60	22.87	4.37
65	0.929	30.50	43.88	2.28
70	0.943	39.40	65.01	1.54
75	0.974	58.50	141.00	0.71
80	1.050	72.00	275.10	0.39

The results obtained for 25° have been calculated to the usual terms adopted for the other compounds and are given in Table No. XXXI. The value for the solubility in water alone is more or less uncertain since the first few drops of the salicylate added to 50 c. c. of water apparently remained undissolved. The opalescence which is produced in the solutions of higher alcoholic concentration by an excess of salicylate is not observed in water alone, and the detection of the end point is therefore uncertain. Determinations of the solubility in water, which are no doubt very accurate, have recently been reported by Gibbs.[a] They were made by agitating an excess of the salicylate with pure water for eighteen hours or longer and analyzing the clear solution by a colorimetric method. The result for 30° was 0.074 grams $C_6H_4OHCOOCH_3$ per 100 cubic centimeters H_2O. From the present results it is seen that very little increase of solubility of methyl salicylate occurs even up to approximately 30 per cent alcohol. Beyond this point the increase is more rapid, and between 60 and 80 per cent alcohol the curve (see figure 6) turns up very abruptly, evidently reaching the point corresponding to complete miscibility of the methyl salicylate at about 85 weight per cent alcohol. There are no quantitative figures for the solubility of methyl salicylate in alcohol to be found in the U. S. Pharmacopœia or indeed in the chemical literature. The available statements are, to the effect that it is slightly soluble in water and in all proportions in alcohol. The results show that the first part of this statement is true not only for water but even for alcoholic solutions up to about 40 per cent strength. The complete miscibility, on the other hand, applies for alcoholic solvents of only about 85 weight per cent or more, and below this strength the amount dissolved diminishes very rapidly, with lowering of alcoholic strength.

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[a] Philippine Jour. of Sci. (A), **3**, 359, 1908.

TABLE No. XXXII.—*Solubility of methyl salicylate in aqueous alcohol solutions at different temperatures.*

EXPERIMENTAL DETERMINATIONS.

Per cent by weight of C_2H_5OH in solvent.	$C_6H_4OHCOOCH_3$ per 100 c. c. alcoholic solvent at—			
	15°.	20°.	25°.	30°.
	c. c.	*c. c.*	*c. c.*	*c. c.*
0.0	(Ca.) 0.1	0.1	0.1	0.1
32.0	0.3	0.4	0.5	0.6
51.0	3.4	4.1	5.3	6.2
70.2	22.8	27.4	34.0	43.2
81.4	160.0	214.0	270.0	335.0

INTERPOLATED RESULTS.

The above determinations plotted on cross-section paper yielded curves from which the following values were obtained:

Per cent by weight of C_2H_5OH in solvent.	$C_6H_4OHCOOCH_3$ per 100 c. c. alcoholic solvent at—			
	15°.	20°.	25°.	30°.
	c. c.	*c. c.*	*c. c.*	*c. c.*
0	(Ca.) 0.1	0.1	0.1	0.1
30	0.3	0.4	0.5	0.6
40	0.8	1.1	1.4	1.8
50	2.4	3.5	5.0	6.0
55	4.2	6.0	7.8	9.5
60	7.7	10.0	12.5	15.5
65	13.0	16.5	20.2	24.5
70	22.0	28.0	33.0	40.0
75	43.0	52.0	62.0	72.0
80	92.0	135.0	180.0	230.0

Another point of much importance in connection with this compound is the great effect of temperature upon the solubility. The variations between 15° and 30° are shown very strikingly by the results contained in Table No. XXXII. It will be noticed that at nearly every concentration of alcohol the amount of methyl salicylate dissolved is more than doubled through the range of 15° of temperature.

The solubility of phenyl salicylate in aqueous alcohol solutions. The sample used for the solubility determinations was analyzed by saponifying in a closed flask and titrating the excess of alkali used. The results showed the material to be of practically 100 per cent purity.

Only one series of solubility determinations was made, the time of shaking being two days. The amount of phenyl salicylate was determined by evaporation of the solvents at 55–60° and drying the residues to constant weight in a vacuum desiccator. The results are given in Table No. XXXIII and the curve in figure 6.

The amount dissolved by water according to the present determination is only about one-third that reported by the pharmacopœia. The actual amount, however, in both cases is very small and the statement of most of the other reference books that salol is almost insoluble in water is perhaps sufficient for all practical purposes. In regard to the figures quoted for alcohol very great variations exist in the literature. Thus it is given as 1 part in 5 by the U. S. Pharmacopœia, as 1 part in 10 by most of the German authorities, and as 1 part in 20 by Squire's Companion to the British Pharmacopœia. The present results show that the value quoted by the U. S. Pharmacopœia is very near the correct figure.

TABLE No. XXXIII.—*Solubility of phenyl salicylate in aqueous alcohol at 25° C.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25° C.	C_6H_4OH-$COOC_6H_5$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	0.999	0.015
0.986	8.9	0.985	0.018
0.954	32.0	0.950	0.067
0.916	51.0	0.912	0.861
0.872	70.2	0.877	4.44
0.828	88.0	0.863	11.91
0.805	96.3	0.878	26.68
0.794	99.8	0.897	34.73

CALCULATED DETERMINATIONS.

The above results plotted on cross-section paper yielded a curve from which the following results were read:

Per cent by weight of C_6H_6OH in solvent.	Specific gravity saturated solution at 25° C.	$C_6H_4OHCOOC_6H_5$ per 100 grams.		Solvent to dissolve 1 gram C_6H_4-$OHCOOC_6H_5$.
		Saturated solution.	Solvent.	
Per cent.		*Grams.*	*Grams.*	*Grams.*
0	0.999	0.015	0.015	6665.00
20	0.967	0.020	0.020	5000.00
40	0.934	0.220	0.220	453.50
50	0.914	0.760	0.770	130.60
60	0.895	2.100	2.150	46.62
70	0.877	4.400	4.600	21.73
80	0.863	7.700	8.340	11.99
90	0.865	14.000	16.280	6.14
[a] 92.3	0.868	17.700	21.510	4.65
100	0.898	35.000	53.860	1.86

[a] U. S. Pharmacopœia strength.

The solubility of quinine salicylate in aqueous alcohol solutions.—The material used for these solubility determinations bore the label "Quinine salicylate, Merck," and consisted of very light fibrous white crystals which melted at approximately 195°. The U. S. Pharmacopœia gives the melting point as 183° to 187° with decomposition. No other tests for the identification or purity of the product were made. The solubility determinations were carried out in the usual manner, the solvents being evaporated and the residues dried in a vacuum desiccator at room temperature. The results are given in Table No. XXXIV and the curve in figure 6.

The curve shows a maximum point at about 90 weight per cent alcohol. The values for the solubility in water and in alcohol of U. S. Pharmacopœia strength differ considerably from those quoted in the U. S. Pharmacopœia; the present results being 1 part in 1,538 and 20.65, respectively, for water and alcohol, and those of the Pharmacopœia being 1 part in 77 and 1 part in 11.

As is generally the case with the solubilities of alkaloids and their salts, the values quoted in the literature for quinine salicylate are very variable. Thus, for the solubility in water, figures are given all the way from 1 part in 77 parts to 1 part in 630 parts of water, and for alcohol from 1 part in 11 to 1 part in 25. Such great differences can not be satisfactorily explained, and, as has been mentioned in the first part of this bulletin, the subject of the solubilities of the alkaloids is one upon which there is the most pressing need for investigation.

TABLE NO. XXXIV.—*Solubility of quinine salicylate in aqueous alcohol solutions at 25° C.*

EXPERIMENTAL RESULTS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_6H_4OHCOOH.C_{20}H_{24}N_2O_2+\frac{1}{2}H_2O$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.00	0.999	0.065
0.954	32.40	0.950	0.571
0.915	51.60	0.914	1.839
0.869	71.50	0.873	3.390
0.815	92.60	0.825	4.609
0.804	96.60	0.811	4.066
0.794	99.90	0.797	3.152

TABLE NO. XXXIV.—*Solubility of quinine salicylate in aqueous alcohol solutions at 25° C.*—Continued.

CALCULATED RESULTS.

The above results plotted on cross-section paper yielded a curve from which the following figures were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	Quinine salicylate per 100 grams.		Solvent to dissolve 1 gram quinine salicylate.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	0.999	0.065	0.065	1,538.00
10.0	0.982	0.080	0.080	1,250.00
20.0	0.966	0.200	0.200	500.00
30.0	0.952	0.480	0.480	207.00
40.0	0.935	1.000	1.010	99.00
50.0	0.916	1.700	1.730	57.80
60.0	0.896	2.450	2.510	39.80
70.0	0.876	3.270	3.380	29.60
80.0	0.854	4.200	4.380	22.80
90.0	0.832	4.710	4.940	20.20
[a] 92.3	0.826	4.620	4.840	20.65
100.0	0.797	3.150	3.250	30.75

[a] U. S. Pharmacopœia strength.

Solubility of sodium salicylate in aqueous alcohol solutions.—The sodium salicylate which was used for the solubility determinations given herewith was carefully recrystallized from hot 95 per cent alcohol and dried for about a week in a vacuum desiccator containing concentrated H_2SO_4. The scaly crystals were almost white, and an analysis by the pharmacopœial method, modified as already described under sodium benzoate, gave results indicating a purity of 100.2 per cent. Two closely agreeing series of solubility determinations were made; in the first the time of shaking was two days and in the second five days. The weighed portions of the saturated solutions were transferred to weighing bottles and the solvents evaporated at not over 60° C. The residues were dried to constant weight in a vacuum desiccator containing concentrated sulphuric acid. The results are given in Table No. XXXV and the curve is shown in figure 6.

It will be noted, as might be expected, that alcohol decreases regularly the solubility of sodium salicylate. The decrease is at first very gradual and then more rapid, giving a curve which bows upward and shows that the solubility in mixtures of alcohol and water is greater than corresponds to a simple additive function of the amount dissolved in each solvent separately.

The values in the literature for water and pharmacopœial alcohol agree on the whole fairly well with the results here shown.

TABLE NO. XXXV.—*Solubility of sodium salicylate in aqueous alcohol solutions at 25° C.*

EXPERIMENTAL RESULTS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_6H_4OHCOONa$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.252	53.56
0.986	8.9	Not det.	52.10
0.954	32.4	1.169	47.40
0.915	51.6	1.102	41.93
0.869	71.5	1.010	32.10
0.815	92.6	0.864	11.75
0.804	96.6	0.828	6.56
0.794	99.9	0.805	3.82

CALCULATED DETERMINATIONS.

The above results plotted on cross-section paper gave a curve from which the following figures were read or calculated:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$C_6H_4OHCOONa$ per 100 grams.		Solvent to dissolve 1 gram C_6H_4-$OHCOONa$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.256	53.56	115.30	0.867
10.0	1.235	52.10	108.70	0.919
20.0	1.205	50.20	100.80	0.992
30.0	1.176	48.00	92.30	1.083
40.0	1.142	45.50	83.50	1.198
50.0	1.106	42.20	73.00	1.370
60.0	1.060	38.40	62.30	1.604
70.0	1.016	33.00	49.20	2.031
80.0	0.957	25.00	33.30	3.000
90.0	0.885	15.00	17.60	5.666
[a] 92.3	0.864	12.00	13.60	7.333
100.0	0.805	3.82	3.98	25.160

[a] U. S. Pharmacopœia strength.

Solubility of strontium salicylate in aqueous alcohol solutions.—The averages of the results obtained from two samples of strontium salicylate purchased for the following solubility determinations were, respectively, 99.04 and 99.55 per cent $(C_6H_4OHCOO)_2Sr+2H_2O$; the analyses being made according to the U. S. Pharmacopœia method, which involves incineration of the sample after mixing with concentrated sulphuric acid and repeating this procedure until a residue of strontium sulphate uncontaminated with unburned carbon is obtained. The method yields satisfactory results, but great care is required to prevent loss by spattering and to insure the complete removal of all carbon. The former source of error may be satisfactorily overcome by placing the crucible in a hole in an asbestos board and covering with a piece of ashless filter paper during the evaporation of the excess of sulphuric acid, the filter paper being allowed to drop into the crucible when the temperature is raised to redness.

The better of the above-mentioned samples of strontium salicylate was recrystallized from hot 80 to 90 per cent alcohol and the crystals dried in a vacuum desiccator over concentrated sulphuric acid for about a week. The product analyzed practically 100 per cent $(C_6H_4OHCOO)_2Sr+2H_2O$, showing that no loss of water of crystallization had occurred in drying the material as described.

The tubes for the solubility determinations were prepared as has already been described, but after the period of rotation it was noticed that the solid phase in the tube containing the solvent of 99.9 per cent alcoholic strength had been converted into an amorphous bulky white powder. It therefore appeared that absolute alcohol is able to remove some or all of the water of crystallization of strontium salicylate. This point was tested by preparing a quantity of the dehydrated salt by allowing some of the crystallized material to stand in contact with absolute alcohol until it had practically all been converted to the powder form and then filtering, washing, and drying. Solubility determinations made with this product did not differ appreciably from those obtained for the crystalline dihydrate. Analyses of it gave an amount of strontium sulphate which corresponded to 93.87 per cent $(C_6H_4OHCOO)_2Sr$, 99.49 per cent of monohydrate or 100.85 per cent of $(C_6H_4OHCOO)_2Sr+1\frac{1}{2}H_2O$. These results show that although absolute alcohol changes the appearance of the ordinary strontium salicylate very materially the amount of dehydration which it effects is comparatively small. Under the high power microscope the salicylate powder shows minute irregular crystals and no appreciable amount of amorphous material.

Four series of solubility determinations were made, in three of which the crystalline salicylate was used, and in the other the powder obtained by treating the crystals with absolute alcohol. The results in all cases were in satisfactory agreement.

TABLE No. XXXVI.—*Solubility of strontium salicylate in aqueous ethyl alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$(C_6H_4OHCOO)_2Sr+2H_2O$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.022	5.04
0.986	8.9	1.007	4.91
0.954	32.4	0.979	6.55
0.915	51.6	0.945	8.02
0.869	71.5	0.891	5.80
0.804	96.6	0.804	1.24
0.794	99.9	0.790	0.44

TABLE NO. XXXVI.—*Solubility of strontium salicylate in aqueous ethyl alcohol solutions at 25°.*—Continued.

CALCULATED DETERMINATIONS.

These results plotted on cross-section paper gave a curve from which the following figures were read or calculated:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$(C_6H_4OHCOO)_2Sr+2H_2O$ per 100 grams.		Solvent to dissolve 1 gram of $(C_6H_4OHCOO)_2Sr+2H_2O$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.022	5.04	5.31	18.85
10.0	1.006	4.88	5.13	19.49
20.0	0.993	5.22	5.51	18.16
30.0	0.982	6.20	6.61	15.13
40.0	0.966	7.70	8.34	11.99
50.0	0.948	8.08	8.79	11.38
60.0	0.923	7.15	7.70	12.98
70.0	0.893	5.90	6.27	15.95
80.0	0.859	4.40	4.60	21.73
90.0	0.824	2.56	2.63	38.07
[a]92.3	0.815	2.02	2.06	48.51
100.0	0.790	0.44	0.44	226.20

[a] U. S. Pharmacopœia strength.

Although in the present case we are dealing with a salt containing water of crystallization and naturally expect that in so far as the salt dissolves, this water will change the concentration of the alcoholic solvent, it happens that this error is really not large enough to materially affect the results. If we consider the solution in which the maximum amount of salt is dissolved it is found that the amount of water arising from this salt is less than 1 per cent of the weight of the solvent required to dissolve this quantity of the salt. This solvent is the one containing approximately 50 weight per cent of alcohol, hence it is evident that the maximum error produced is less than one-half of 1 per cent and in the present case this amount would not affect the position of the solubility curve obtained.

The analyses of the saturated solutions were made both by weighing the evaporated residues which were dried to constant weight in a vacuum desiccator containing concentrated sulphuric acid and also by determination of the strontium in these residues by incineration as already described. The two methods gave satisfactory agreement. The results which were obtained have been compiled in the first part of Table No. XXXVI. The curve drawn through the plotted results gave the figures shown in the lower part of the table.

The solubility curve for this salt which is shown in figure 6 is quite remarkable, and the cause for its irregularities can not be readily explained. The solubility at first decreases slightly and then rises very rapidly to a sharp maximum of 8.1 grams in 100 grams of 48 weight per cent alcohol. After which with increasing alcoholic strength the solubility falls steadily to 0.44 gram of salicylate in 100 grams of absolute alcohol.

The results for the solubility of strontium salicylate quoted by the U. S. Pharmacopœia are respectively 1 part in 18 parts of water and 1 part in 66 parts of alcohol. The present results are, however, 1 part in 18.85 and 1 part in 48.51.

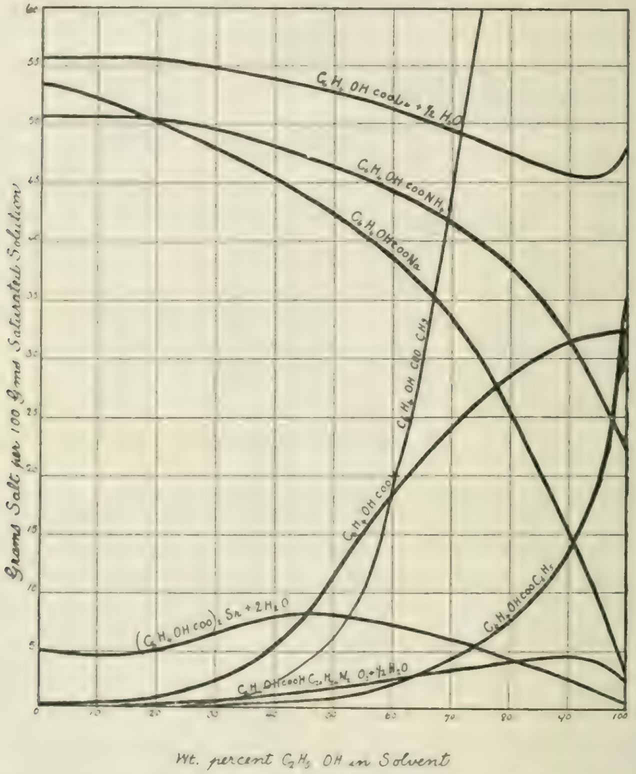

FIG. 6.—Curves showing the solubilities of salicylic acid and the salicylates in aqueous alcohol solutions at 25°.

STEARIC ACID.

Solubility of stearic acid in aqueous alcohol solutions.—No definite requirements of purity of this acid are prescribed by the Pharmacopœia and likewise no quantitative methods for its analysis. It would really appear that commercial more or less impure material obtained from the more solid fats, chiefly tallow, is all that the Pharmacopœia demands. It is clear therefore that only approximate solubility values are necessary for such a product. The material used for the present solubility determinations was purchased as C. P. and is presumably the best that the market affords. A quantitative determination made by dissolving the weighed sample in neutralized alcohol and titrating aliquot portions of the solution with standard alcoholic potassium hydroxide, using phenolphthaleine as indicator, gave results corresponding to 102.3 per cent $C_{17}H_{35}COOH$ in the case of a sample from one source and 101.7 per cent with a different sample of the acid. It was noticed that in making these titrations the presence of water increased the amount of alkali required and therefore led to high results. Thus titrations made in alcoholic solutions containing about 50 per cent alcohol might give results 1 per cent or more above that found with solutions in which only relatively very little water was present.

The analytical results upon these two samples indicate that they were really not pure stearic acid, but they are no doubt much within the very loose requirements of the pharmacopœia, and the accompanying solubility results will show the general direction and approximate solubility values, but not the absolute figures for the purest stearic acid.

Table No. XXXVII.—*Solubility of stearic acid in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_{17}H_{35}COOH$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	0.999	0.034
0.9752	17.0	0.972	0.035
0.9359	41.7	0.929	0.121
0.8718	70.2	0.864	0.813
0.8234	89.6	0.817	3.163
0.8048	96.4	0.803	6.442
0.7941	99.9	0.795	8.277

CALCULATED RESULTS.

The above figures plotted on cross-section paper gave a curve from which the following values were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$C_{17}H_{35}COOH$ per 100 grams.		Solvent to dissolve 1 gram $C_{17}H_{35}COOH$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	0.999	0.034	0.035	2,809.0
20.0	0.967	0.04	0.04	2,500.0
40.0	0.932	0.10	0.10	999.0
50.0	0.911	0.18	0.18	555.0
60.0	0.888	0.40	0.40	249.0
70.0	0.865	0.80	0.81	124.0
80.0	0.841	1.63	1.66	60.4
90.0	0.818	3.30	3.41	29.3
92.3	0.813	4.15	4.33	23.1
95.0	0.807	5.55	5.88	17.0
100.0	0.795	8.30	9.05	11.1

Only one series of determinations was made and the time allowed for saturation was three days. The weighed saturated solutions were transferred to Erlenmeyer flasks with the aid of (neutral) absolute alcohol and titrated with standard alcoholic alkali. It was noticed that if, after the titration, water be added to the solutions, the faint excess of pink color was at first discharged and then with more water it reappeared and became deeper red with continued dilution. The titration results were calculated without making a correction for the impurity indicated by the analysis of the samples. The results are given in Table No. XXXVII and the solubility curve shown in figure 4.

An examination of the literature shows the most divergent values for the solubility of stearic acid in alcohol. This is probably due to a number of causes. The variation in the purity of the samples is to be considered first, but perhaps the most serious difficulty is the very steep character of the curve in the vicinity of the very strong alcohol solutions. It will be noticed that for a change of alcoholic strength of from only 90 to 95 per cent the amount of acid dissolved is increased more than 1.7 times; therefore small differences in the concentration of the alcohol used by different investigators make large differences in the results found. Although there are no available results showing the effect of temperature on the solubility of stearic acid, it is probable that this is also a source of some of the differences in the reported values.

Solubility of stearic acid in organic solvents.—These determinations were made by agitating an excess of the acid with the several solvents for two days. The saturated solutions were titrated with standard alcoholic alkali as already mentioned for the solubility determinations in aqueous alcohol. The results are given in Table No. XXXVIII. It will be noticed that stearic acid is fairly soluble in all of the ten organic solvents. The solubility is greatest in ether and least in nitrobenzene.

TABLE NO. XXXVIII.—*Solubility of stearic acid in organic solvents at 25°.*

Solvent.	d of solvent.	d_{25} of saturated solution.	$C_{17}H_{35}COOH$ dissolved per 100.			Solvent to dissolve 1 gram $C_{17}H_{35}COOH$.
			Grams saturated solution.	Grams solvent.	C. C. solvent.	
			Grams.	*Grams.*	*Grams.*	*Grams.*
Acetone	d_{15}=0. 797	0. 815	4. 73	4. 97	3. 96	20. 14
Amyl alcohol (iso)	d_{20}=0. 817	0. 815	9. 43	10. 41	8. 51	9. 60
Amyl acetate	d_{20}=0. 875	0. 867	11. 19	12. 60	11. 03	7. 94
Carbon bisulphide	d_{25}=1. 259	1. 163	19. 20	23. 76	29. 91	4. 21
Carbon tetrachloride	d_{25}=1. 587	1. 465	10. 25	11. 42	18. 13	8. 76
Chloroform	d_{22}=1. 476	1. 332	15. 54	18. 40	27. 16	5. 44
Ether (abs.)	d_{22}=0. 711	0. 744	20. 04	25. 06	17. 82	3. 99
Ethyl acetate	d_{25}=0. 892	0. 895	7. 36	7. 95	7. 09	12. 59
Nitrobenzene	d_{25}=1. 205	1. 199	1. 24	1. 26	1. 52	79. 66
Toluene	d_{15}=0. 872	0. 865	13. 61	15. 75	13. 74	6. 35

TANNIC ACID.

Attempts to determine the solubility in aqueous alcohol solutions of a sample of tannic acid [a] of apparently very good quality were unsuccessful on account of the impossibility of saturating the solution with the acid. So much of the sample dissolved in water and also in absolute alcohol that before an excess of it remained undissolved the solution had become so viscous that it flowed from one end of the tube to the other only with extreme slowness. It appeared doubtful whether it would have been possible to separate the saturated solution from the undissolved material even if the saturation point could have been reached. Furthermore, there was apparently no satisfactory method available for analyzing the saturated solution even if it could have been obtained. It was therefore necessary to abandon attempts to determine the solubility of this acid in the manner followed for the remaining members of the series of pharmacopœial compounds.

According to the literature the solubility in water is given all the way from 1 part in 0.35 parts of water to 1 part in 5.0 parts, and the solubility in alcohol from 1 part in 0.23 to 1 part in 2.0. The statement that it is almost insoluble in absolute alcohol occurring in several reference books is, according to the experiment mentioned above, certainly open to question.

TARTARIC ACID AND THE TARTRATES.

Solubility of tartaric acid in aqueous alcohol solutions.—The sample used for the solubility determination was analyzed by titration with normal alkali, using phenolphthaleine as indicator. The results corresponded to a purity of 99.3 per cent $C_2H_2(OH)_2(COOH)_2$. The melting point was found to be 166.1° to 167.3° (cor.).

One series of determinations was made and the time allowed for saturation, with constant shaking, was two days. The saturated solutions were weighed and, after dilution, aliquot portions were titrated with normal alkali. The results are given in Table No. XXXIX and the curve shown in figure 7. The decrease in solubility with increase of alcoholic content of the solution is perfectly regular, but at no point is the sum of the amounts which the alcohol and water in each solvent would dissolve independently as great as actually found. The figure for water agrees fairly well with that quoted by the pharmacopœia, but with alcohol of U. S. Pharmacopœia strength the difference is much greater.

A series of determinations of the solubility of tartaric acid in water at different temperatures published by Leidie [b] contains a value for

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[a] Kindly furnished by Dr. Geo. W. Hoover, of the Drug Laboratory, Bureau of Chemistry, U. S. Department of Agriculture.

[b] Compt. rend., **95,** 87, 1882.

25°, which is considerably above the present results, viz, 147.44 grams acid per 100 grams water instead of 137.5. Other values from the principal pharmaceutical reference books for the temperature 15° also indicate that Leidie's results are too high for this temperature. Since he gives no details concerning the purity of the samples employed or of the method by which his determinations were made, satisfactory conclusions in regard to the accuracy of his results can not be drawn. It is probable, however, that the material at hand and the facilities for accurate determinations available nearly thirty years ago were not as satisfactory as we have at present, and therefore his results should not be accepted with the same confidence that is to be placed in the more recent determinations.

TABLE NO. XXXIX. *Solubility of tartaric acid in aqueous alcohol solutions at 25° C.*

EXPERIMENTAL RESULTS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_2H_2(OH)_2(COOH)_2$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.321	57.93
0.9856	8.9	1.304	56.10
0.9545	32.0	1.246	51.40
0.9164	51.0	1.181	46.76
0.8718	70.2	1.091	39.95
0.8190	91.4	0.963	27.83
0.8048	96.3	0.929	24.22
0.7941	99.9	0.906	21.67

CALCULATED RESULTS.

The above figures plotted on cross-section paper gave a curve from which the following values were read and calculated:

Per cent by weight of C_2H_5OH in solvent.	d_{25} of saturated solution.	$C_2H_2(OH)_2(COOH)_2$ per 100 grams.		Solvent to dissolve 1 gram $C_2H_2(OH)_2(COOH)_2$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.321	57.9	137.5	0.727
10.0	1.300	56.0	127.3	0.786
20.0	1.276	54.1	117.9	0.848
30.0	1.251	52.0	108.3	0.923
40.0	1.220	49.6	98.4	1.016
50.0	1.184	47.0	88.6	1.128
60.0	1.142	43.9	78.3	1.277
70.0	1.095	40.2	66.9	1.495
80.0	1.040	35.3	54.6	1.832
90.0	0.973	29.0	40.8	2.448
[a] 92.3	0.955	27.2	37.4	2.676
95.0	0.937	25.4	34.1	2.937
100.0	0.905	21.6	27.6	3.629

[a] U. S. Pharmacopæia strength.

Solubility of tartaric acid in organic solvents at 25°.—The solubility in the several solvents was determined in the manner already described, the dissolved acid being estimated by the titration method. The results are given in Table No. XL, and show that, aside from amyl alcohol and ether, none of the six solvents dissolve much more than traces of the acid. Of these solvents quantitative results for ether alone are to be found in the literature. Bourgoin[a] found that 100 grams of absolute ether dissolved 0.40 grams of tartaric acid at 15°. As compared with the present value for 25°, it appears as though Bourgoin's figure is somewhat too low; but this can not be stated positively.

TABLE NO. XL.—*Solubility of tartaric acid in organic solvents at 25°.*

Solvent.	d of solvent.	d_{25} of saturated solution.	$C_2H_2(OH)_2(COOH)_2$ per 100.			Solvent to dissolve 1 gram $C_2H_2(OH)_2$ $(COOH)_2$.
			Grams saturated solution.	Grams solvent.	C. C. solvent.	
			Grams.	*Grams.*	*Grams.*	*Grams.*
Amyl alcohol (iso)	d_{20}=0.817	0.824	3.383	3.50	2.86	28.6
Benzene	d_{25}=0.873	0.875	0.0086	0.0086	0.0075	11620.0
Carbon tetra-chloride	d_{25}=1.587	1.589	0.0189	0.0189	0.030	5289.0
Chloroform	d_{22}=1.476	....	Trace.	Trace.	Trace.	∞
Ether	d_{22}=0.711	0.715	0.6096	0.6134	0.4361	163.0
Toluene	d_{15}=0.872	0.865	Trace.	Trace.	Trace.	∞

Solubility of antimony potassium tartrate in aqueous alcohol solutions.—The sample used for the following solubility determinations was analyzed by the iodometric method recommended by the pharmacopœia. Two grams of the sample were dissolved in water and diluted to 200 cubic centimeters, 50 cubic centimeter portions of the solution to which 25 cubic centimeters of saturated sodium bicarbonate solution were added required 30.3 cubic centimeters 0.1 N iodine, therefore indicating a purity of 100.6 per cent $C_2H_2(OH)_2(COOK)(COOSbO) + \frac{1}{2}H_2O$. The addition of alcohol to the solution before titration with the iodine did not affect the results, showing that the method is applicable to the determination of the tartrate dissolved in the alcohol solutions used for the solubility determinations.

One series of determinations was made, the time allowed for the saturation being two days. The titrations of the dissolved tartrate being made, as above mentioned, for the sample. The results are given in Table No. XLI and the curve shown in figure 7.

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[a] Ann. chim. phys. (5), **13**, 405, 1878.

TABLE No. XLI.—*Solubility of antimony potassium tartrate in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_2H_2(OH)_2$ (COOK) (COOSbO) $+\frac{1}{2}H_2O$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.052	7.85
0.9856	8.9	1.011	4.20
0.9752	17.0	0.988	2.49
0.9628	26.4	0.966	1.16
0.9164	51.0	0.911	0.22
0.8718	70.2	0.865	0.06
0.8234	89.6	0.816	Trace.
0.7941	99.9	0.788	Trace.

CALCULATED RESULTS.

The above figures plotted on cross-section paper gave a curve from which the following results were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$C_2H_2(OH)_2(COOK)(COOSbO)+\frac{1}{2}H_2O$ per 100 grams.		Solvent to dissolve 1 gram $C_2H_2(OH)_2$ (COOK) (COOSbO) $+\frac{1}{2}H_2O$.
		Saturated solution.	Solvent.	
		Grams.		*Grams.*
0	1.052	7.85	8.52	11.74
5	1.025	5.50	5.82	17.18
10	1.007	3.92	4.08	24.51
20	0.980	1.92	1.96	51.09
30	0.958	0.84	0.85	118.0
40	0.935	0.38	0.38	262.2
50	0.913	0.23	0.23	433.8
60	0.890	0.12	0.12	832.2
70	0.866	0.06	0.06	1665.0
100	0.788	Trace.	Trace.	∝

The above results show a somewhat greater solubility of the tartrate in water than is quoted by the U. S. Pharmacopœia, the respective values per 100 grams of water being 6.45 and 8.52. Since the solubility quoted by most of the pharmaceutical reference books in which the temperature standard is 15° agrees so closely with the value quoted by the U. S. Pharmacopœia, it would appear that the figure in the latter book was taken directly from the others without regard to the difference in temperature standards.

Solubility of potassium bitartrate in aqueous alcohol solutions.—The pharmacopœia recommends a quantitative method based upon the ignition of the sample and titrating the residue with standard acid using methyl orange as indicator. Since, however, the compound contains an available hydrogen ion which can be titrated directly with standard alkali, an ignition as recommended by the pharmacopœia appears altogether unnecessary. Furthermore, experiments showed that decrepitation occurred during the ignition and low results were apt to be obtained. An analysis of the sample used for the following solubility determinations showed a purity of 99.3 per cent $C_2H_2(OH)_2(COOH)(COOK)$ by direct titration with standard alkali using phenolphthaleine as indicator, but only 97.2 per cent was found by the ignition method; since, however, decrepitation occurred, it is easy to explain the low result by the latter method.

One series of solubility determinations was made, the time of agitation being two days. The weighed saturated solutions were titrated with 0.1 N sodium hydroxide, using phenolphthaleine as indicator. On account of the comparatively small amounts of dissolved tartrate it was necessary to apply corrections to the titrations for the acidity of the alcoholic solvents. The actual amount of the correction was of course very small and would be entirely negligible in such a case as tartaric acid, for instance, where very large amounts are dissolved, but with potassium bitartrate in the stronger alcoholic solvents the correction may amount to one-third of the total amount of the alkali required. The results are given in Table No. XLII and the curve in figure 7.

TABLE NO. XLII.—*Solubility of potassium bitartrate in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_2H_2(OH)_2(COOH)(COOK)$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.002	0.649
0.9856	8.9	0.986	0.382
0.9752	17.0	0.975	0.242
0.9628	26.4	0.961	0.157
0.9164	51.0	0.911	0.062
0.8234	89.6	0.816	0.018
0.7941	99.9	0.789	0.010

CALCULATED RESULTS.

The above figures plotted on cross-section paper gave a curve from which the following results were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$C_2H_2(OH)_2(COOH)(COOK)$ per 100 grams.		Solvent to dissolve 1 gram $C_2H_2(OH)_2(COOH)(COOK)$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.002	0.649	0.654	153.1
10.0	0.985	0.358	0.359	278.4
20.0	0.970	0.210	0.210	475.2
30.0	0.953	0.131	0.131	762.3
40.0	0.933	0.087	0.087	1,148.0
50.0	0.912	0.064	0.064	1,562.0
60.0	0.890	0.043	0.043	2,323.0
80.0	0.842	0.023	0.023	4,347.0
92.3	0.807	0.014	0.014	7,144.0
100.0	0.789	0.010	0.010	10,000.0

The present value for the solubility in water is in close agreement with the results of Noyes and Clement,[a] which is 0.654 gram per 100 grams saturated solution, lending weight, therefore, to the correctness of this figure and indicating the probable inaccuracy of the results of Roelofsen[b] and Blarez.[c] The solubility of potassium bitartrate in aqueous alcohol solutions was also determined by Roelofsen at several temperatures. The results were more or less irregular, however, and are evidently only approximately correct. The method he used for obtaining saturation is certainly of doubtful efficacy. Various amounts of 93 weight per cent alcohol were added to the saturated aqueous solution of the salt and the solutions shaken at intervals during about six hours while kept at the desired temperature. Some experiments made by me with ammonium iodide by a similar procedure involving shaking at intervals daily for several weeks did not give saturated solutions in the several cases.

The figure quoted by the pharmacopœia for the solubility in water is 1 part in 200, which is evidently too low and was probably taken from some other pharmaceutical reference book in which the temperature standard was 15° instead of 25°.

Solubility of potassium sodium tartrate in aqueous alcohol solutions.—The method given by the pharmacopœia for analyzing this salt depends upon the incineration and subsequent titration of the ash with standard hydrochloric acid, using methyl orange as indicator. This method applied to the sample used for the following solubility determinations gave an average of 99.3 per cent $C_2H_2(OH)_2(COONa)(COOK)+4H_2O$. By evaporating the titrated solution to dryness and determining the potassium as the platinic chloride, 14.15 per cent was found instead of the theoretical 13.86 per cent; the molecular ratio of sodium to potassium as calculated from this determination gave $Na:K=1:1.02$. Several determinations of the molecular ratio by calculation from the weight of sodium and potassium chloride obtained by evaporation and of the total chlorine in the mixed chlorides by titration with standard silver nitrate, using the usual algebraic formula, gave fairly satisfactory results. In this determination, as well as by the direct estimation of the potassium, very small differences in the analytical results make quite large errors in the molecular ratio, consequently this determination is not of very great value in judging the quality of samples of this salt.

One series of solubility determinations was made and the time allowed for saturation was five days. The saturated solutions were weighed and transferred to weighing bottles, but satisfactory residues for weighing could not be obtained upon evaporation, and it was therefore necessary to redissolve, ignite in platinum dishes, and titrate the ash with standard acid. The usual precautions for obtaining all of the alkali in solution for titration as described under sodium benzoate (p. 28) were of course observed. The results were all calculated to the hydrated salt, since no change in the solid phase was observed in any of the solutions except those with 81.5 and 99.9 weight per cent alcohol, in which almost inappreciable amounts of the salt were dissolved. The results are given in Table No. XLIII and the curve shown in figure 7.

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[a] Z. physik. Chem., **13,** 413, 1894.

[b] Am. Chem. Jour., **16,** 466, 1894.

[c] Compt. rend., **112,** 434, 1891.

TABLE NO. XLIII.—*Solubility of potassium sodium tartrate in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$C_2H_2(OH)_2$ (COONa) (COOK) $+4H_2O$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O	0.0	1.310	53.33
0.9856	8.9	1.229	43.26
0.9752	17.0	1.152	31.49
0.9628	26.4	1.065	17.63
0.9359	41.7	0.955	5.09
0.9164	51.0	0.922	2.20
0.8441	81.5	0.838	0.04
0.7941	99.9	0.789	Trace.

CALCULATED RESULTS.

The above figures plotted on cross-section paper gave a curve from which the following results were obtained:

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$C_2H_2(OH)_2$(COONa) (COOK) $+ 4H_2O$ per 100 grams.		Solvent to dissolve 1 gram, $C_2H_2(OH)_2$ (COONa) (COOK) $+4H_2O$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0	1.310	53.33	114.20	0.875
10	1.216	41.60	71.20	1.404
20	1.124	26.20	35.50	2.820
30	1.034	13.80	16.00	6.250
40	0.961	6.00	6.40	15.660
50	0.908	2.40	2.50	40.700
60	0.878	0.90	0.90	110.100
70	0.857	0.30	0.30	332.400
80	0.840	0.06	0.06	1,665.000
100	0.789	Trace.	Trace.	∞

It will be noticed that the U. S. Pharmacopœia figure for the solubility in water is much too low, evidently having been taken from some other reference book in which the temperature standard is 15°

instead of 25°. A determination at 15° made by Greenish and Smith[a] is 1 gram $C_2H_2(OH)_2(COONa)(COOK)+4H_2O$ in 1.392 cubic centimeters H_2O or 71.84 grams of the salt per 100 grams H_2O.

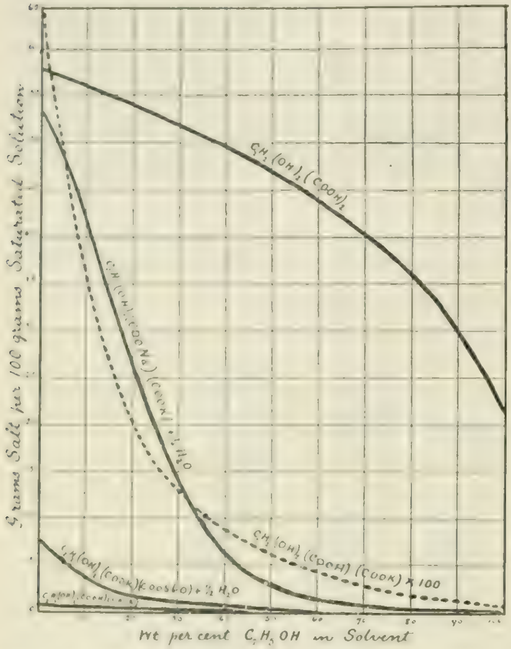

FIG. 7. Curves showing the solubilities of tartaric acid and the tartrates in aqueous alcohol solutions at 25°.

TRICHLORACETIC ACID.

No quantitative figures for the solubility of this acid are to be found in the literature. The general statement that it is very soluble in water and alcohol is widely quoted. The compound is in fact very deliquescent; a crystal exposed in the air quickly absorbs enough moisture to cause it to liquify. It would probably be found one of the most efficient dehydrating agents.

Two samples which were analyzed by titrating aliquot portions of the aqueous solutions of weighed amounts were found to contain, respectively, 97.7 and 98.6 per cent CCl_3COOH. Standard 0.1 N NaOH was used and phenolphthaleine employed as indicator. The end point was very sharp, but the pink color was inclined to fade on standing. The sample which gave the 98.6 per cent results was from a fresh bottle just opened; the other had been previously opened and partially used.

In making the solubility determinations 25 cubic centimeter glass stoppered cylinders were about half filled as quickly as possible with the acid and 2 to 3 cubic centimeter portions of the solvents which were water, 32, 70.2, and 99.9 weight per cent alcohol, added. After a short period of rotation the excess of solid phase had disappeared in the cylinders to which the alcoholic solvents had been added, more of the acid was therefore introduced into these and this addition of acid repeated from time to time without yielding a saturated solution in any case except the cylinder in which water alone was the solvent. After about 15 hours constant agitation at 25°, the saturated solution in water was removed and after weighing in the pycnometer the amount of the trichloracetic acid determined by titration. The following results were obtained:

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[a] Pharm. Jour. (Lond.), June 22, 1901.

Solubility of trichloracetic acid in water at 25°.

Specific gravity of saturated solution at 25°	1. 615
Grams CCl_3COOH per 100 grams saturated solution	92. 32
Grams CCl_3COOH per 100 grams water	1, 201. 00
Grams H_2O to dissolve 1 gram CCl_3COOH	0. 0832

Since my supply of trichloracetic acid was nearly exhausted before either of the alcoholic solutions was saturated at 25°, I lowered the temperature of the bath to 15° and hoped that an additional crystal of trichloracetic acid in each cylinder would cause the separation of the excess of acid which might possibly be present at the lower temperature. The added crystals dissolved, however, as quickly as before and there was no further possibility of obtaining saturated solutions. Even the aqueous solution to which a little more water had been added contained none of the undissolved acid. Although none of the four solutions were saturated at 15°, I thought it would nevertheless be of interest to determine the quantity of acid present in each and thus have figures which would show that the actual solubility was not below the amounts found. In the case of the aqueous solution the following results were obtained:

Specific gravity of solution (not quite saturated) at 15°	1. 593
Grams CCl_3COOH per 100 grams of the solution	87. 22

Thus it is certain that the solubility of trichloracetic acid in water at 15° is *not less* than 87.22 grams per 100 grams of the saturated solution.

Now, in the case of the solution made with trichloracetic acid and alcohol of 99.9 weight per cent the specific gravity was found to be 1.508. On diluting 10 cubic centimeters of this solution with water in order to determine the dissolved acid by titration of an aliquot part, a second liquid layer amounting to about 4 cubic centimeters separated. The aqueous part having been diluted to 500 cubic centimeters was found by titration to contain 8.9475 grams CCl_3COOH. It gave no opalescence with silver nitrate, showing that by whatever reaction the second compound had been formed no free hydrochloric acid had been liberated. It appeared most probable that the ethyl ester of the trichloracetic acid had been formed, no doubt under the influence of the very great dehydrating power of the acid. That the second liquid phase was really the trichloracetic acid ester was proved by separating and determining its boiling point which was found to be 162° to 165°. The boiling point as given by Beilstein is 164°. The liquid obtained therefore by attempting to prepare a saturated solution of trichloracetic acid in absolute alcohol was really a mixture of the trichloracetic acid ethyl ester and trichloracetic acid containing a little water. In adding water to this mixture a separation occurred at a certain point and it therefore appeared of interest to determine the amount of water necessary to just cause this separation and also the amount required to dissolve all of the acid out of the ester solution. The experiment was made as follows: Ten cubic centimeters of the solution was transferred to a graduated cylinder and water added slowly from a burette. It was found that 5.9 cubic centimeters of H_2O just produced a faint opalescence at 15°, 6.05 cubic centimeters gave a milky solution which showed no tendency to separate into two layers until 7.1 cubic centimeters of H_2O had been added and then on standing the two layers measured respectively 9.2 cubic centimeters for the lower and 7.6 cubic centimeters for the upper. Now, by adding more water and shaking, the volume of the lower layer gradually diminished, so that with a total of 8.0 cubic centimeters of water the lower layer measured 8.3 cubic centimeters and with 10 cubic centimeters of water it became 7.2 cubic centimeters in volume. At this point an aliquot portion of the upper layer was removed and on titration it was found that 5.104 grams of CCl_3COOH were present in the whole of the upper layer. According to the previous analysis (p. 87) in which an equal volume of the solution had been diluted to 500 cubic centimeters the amount of acid found was 8.9475 grams, therefore in adding 10 cubic centimeters instead of 500 cubic centimeters of water the removal of the trichloracetic acid from the ester was not complete. In order to ascertain how much additional water would be necessary to dissolve out the remaining (8.9475 − 5.104 =) 3.8435 grams of acid, the upper layer was siphoned off as completely as possible from the 7.1 cubic centimeters of lower layer and successive amounts of water were added and the mixture shaken after each addition. It was found that with 3 cubic centimeters of H_2O the lower layer was reduced in volume to 5.4 cubic centimeters, with 8 cubic centimeters it became 4.6 cubic centimeters, and with 18 cubic centimeters it had become 4.0 cubic centimeters. The aqueous layer was then found to contain a total of 3.226 grams of CCl_3COOH, or nearly the calculate amount which should have been recovered.

The two solutions which had been prepared with 70.2 and 32 weight per cent alcohol respectively were also titrated with water as just described for the solution in which 99.9 weight per cent alcohol had been used. The results were as follows:

Five cubic centimeters of the first (the 70.2 per cent alcoholic solution, required 7.3 cubic centimeters of H_2O to produce opalescence; when 9.0 cubic centimeters of H_2O had been added the layers separated within about one-half hour into 1.4 cubic centimeters of lower and 12.1 cubic centimeters of upper layer. With 15 cubic centimeters of H_2O the volume of the lower layer had been reduced to 1.3 cubic centimeters and with 25 cubic centimeters of water it became 1.1 cubic centimeters.

Five cubic centimeters of the second (the 32 per cent alcoholic solution) required 11.3 cubic centimeters of H_2O to become opalescent, and when a total of 20 cubic centimeters H_2O had been added the lower layer measured only about 0.5 cubic centimeter.

These results show that the solubility of trichloracetic acid can not be determined in solutions containing any appreciable amount of alcohol since the ester of the acid will be formed and therefore alter the equilibrium of the system.

It would no doubt be of much interest to determine the limits within which the ester may form and also the extent to which the alcohol is consumed in the reaction but such experiments are hardly within the scope of the present bulletin.

VALERATES.

The solubility of ammonium valerate.—No requirements are made for this salt by the pharmacopœia other than it be kept in well-stoppered bottles. A sample purchased for solubility determinations was in the form of almost colorless, apparently hygroscopic crystals which when analyzed by distillation of the ammonia gave results indicating a composition of $C_4H_9COONH_4 \cdot 2C_4H_9COOH$. Attempts to saturate a series of alcoholic solvents with this salt were unsuccessful. Since no particular composition is required by the pharmacopœia for this compound it appeared useless to prepare samples and make solubility determinations until some definite requirements are made for this salt.

Solubility of zinc valerate in aqueous alcohol solutions. No method is prescribed by the pharmacopœia for the analysis of this salt, the determination of the zinc was therefore made as follows. An aliquot portion of the solution of the weighed sample was heated to the boiling point and sodium carbonate solution added until the precipitation of the zinc as carbonate was complete. After digestion on the steam bath for about an hour the precipitate was filtered on a Gooch crucible, ignited, and weighed as zinc oxide; the results indicated a purity of 97.8 per cent $Zn(C_4H_9COO)_2 + 2H_2O$. Attempts to ignite the sample directly and determine the zinc by weighing the ignited residue gave low results, indicating that loss by volatilization had occurred.

Two series of solubility determinations were made, the period of constant agitation being three days in each case. The saturated solutions were analyzed by precipitation of the zinc as carbonate as above mentioned, and weighing as zinc oxide. The results are given in Table No. XLIV and the curve shown in figure 5. The pharmacopœial values for water and alcohol are quite different from the above results. The difference for alcohol is considerable, the figure being less than half as great as that shown in the table. Only a few results are quoted in the pharmaceutical reference books and none in the chemical literature. Of the available ones the variation is from 1 part in 40 to 1 part in 120 parts of water and from 1 part in 40 to 1 part in 60 of alcohol.

TABLE No. XLIV. *Solubility of zinc valerate in aqueous alcohol solutions at 25°.*

EXPERIMENTAL DETERMINATIONS.

Solvent.		Specific gravity of saturated solution at 25°.	$Zn(C_4H_9COO)_2+2H_2O$ per 100 grams saturated solution.
Specific gravity at 15°.	Per cent by weight of C_2H_5OH.		
			Grams.
Dist. H_2O.	0.0	1.004	1.437
0.9752	17.0	0.976	0.900
0.9628	26.4	0.961	0.723
0.9459	41.7	0.933	0.809
0.9164	51.0	0.914	0.998
0.8441	81.5	0.844	1.841
0.8234	89.6	0.827	3.143
0.8190	91.4	0.826	4.397
0.8125	93.6	0.830	7.148
0.8048	96.3	0.835	10.53
0.7941	99.9	0.844	15.61

The above figures plotted on cross-section paper gave a curve from which the following results were obtained:

CALCULATED RESULTS.

Per cent by weight of C_2H_5OH in solvent.	Specific gravity of saturated solution at 25°.	$Zn(C_4H_9COO)_2+2H_2O$ per 100 grams.		Solvent to dissolve 1 gram. $Zn(C_4H_9COO)_2+2H_2O$.
		Saturated solution.	Solvent.	
		Grams.	*Grams.*	*Grams.*
0.0	1.004	1.44	1.46	68.40
20.0	0.972	0.75	0.75	132.30
40.0	0.936	0.76	0.76	130.60
60.0	0.894	1.15	1.16	86.00
80.0	0.848	1.70	1.73	57.80
85.0	0.836	2.15	2.20	45.50
90.0	0.827	3.20	3.31	30.30
92.5	0.828	5.50	5.82	17.20
95.0	0.832	8.80	9.65	10.36
100.0	0.844	15.60	18.48	5.41

Minimum point is about 30 weight per cent C_2H_5OH; 0.7 grams $Zn(C_4H_9COO)_2+2H_2O$ per 100 grams saturated solution.

The curve is interesting in that it shows a well-marked minimum point at about 30 weight per cent alcohol and also that it changes its direction so abruptly between 85 and 90 weight per cent alcohol. It is possible that a transition of the solid phase occurs at this point and there is really an intersection of two curves, but it did not appear advisable to pursue this point further at the present time.

TABLE No. XLV.—*Showing the solubilities of the pharmacopœial organic acids and their salts in water and official (92.3 weight per cent) alcohol at 25° as compared with the values quoted by the eighth revision of the U. S. Pharmacopœia.*

Compound.	Formula.	Compound per 100 grams solvent at 25°.			
		In water.		In 92.3 weight per cent alcohol.	
		Present result.	U. S. P. value.	Present result.	U. S. P. value.
		Grams.	*Grams.*	*Grams.*	*Grams.*
Ethyl acetate	$CH_3COOC_2H_5$	8.6	11.1	∞	∞
Lead acetate	$(CH_3COO)_2Pb+3H_2O$	72.5	50.0	[a]1.1	3.33
Potassium acetate	CH_3COOK	219.6	250.0	43.9	50.00
Sodium acetate	$CH_3COONa+3H_2O$	125.7	100.0	6.6	4.30
Zinc acetate	$(CH_3COO)_2Zn+2H_2O$	44.5	40.0	4.3	2.77
Benzoic acid	C_6H_5COOH	0.37	0.35	56.74	55.50
Ammonium benzoate	$C_6H_5COONH_4$	22.8	9.5	3.5	3.50
Lithium benzoate	C_6H_5COOLi	38.2	33.3	5.8	7.7
Sodium benzoate	C_6H_5COONa	56.24	62.5	2.04	2.3
Camphoric acid	$C_8H_{14}(COOH)_2$	0.76	0.8	104.5	(b)
Citric acid	$(CH_2)_2COH(COOH)_3+H_2O$	207.70	185.2	116.0	64.5
Bismuth citrate	$(CH_2)_2COH(COO)_3Bi$	0.011	(c)	0.07	(c)
Bismuth and NH_4 citrate	Variable composition	22.25	(d)	0.0	(e)
Lithium citrate	$(CH_2)_2COH(COOLi)_3+4H_2O$	74.5	50.0	0.04	(f)
Potassium citrate	$(CH_2)_2COH(COOK)$	181.8	200.0	0.01+	(e)
Sodium citrate	$(CH_2)_2COH(COONa)+5\frac{1}{2}H_2O$	92.7	90.9	0.0	(g)
Gallic acid	$C_6H_2(OH)_3COOH+H_2O$	1.16	1.18	27.23	24.1
Sodium phenolsulphonate	$C_6H_4(OH)SO_3Na+2H_2O$	24.1	20.8	0.9	0.77
Zinc phenolsulphonate	$(C_6H_4(OH)SO_3)_2Zn+8H_2O$	66.1	59.0	72.1	59.0
Salicylic acid	$C_6H_4OHCOOH$	0.22	0.32	46.85	50.0
Ammonium salicylate	$C_6H_4OHCOONH_4$	103.2	111.0	42.86	43.4
Bismuth subsalicylate	$C_6H_4OHCOO\cdot OBi$	0.01	(f)	0.105	....
Lithium salicylate	$C_6H_4OHCOOLi+\frac{1}{2}H_2O$	127.3	(d)	83.8	(d)
Methyl salicylate	$C_6H_4OHCOOCH_3$	0.1	(e)	∞	∞
Phenyl salicylate	$C_6H_4OHCOOC_6H_5$	0.01+	0.043	21.51	20.0
Quinine salicylate	$C_6H_4OHCOOHC_{20}H_{24}N_2O_2+\frac{1}{2}H_2O$.	0.06+	1.3	4.84	9.0
Sodium salicylate	$C_6H_4OHCOONa$	115.3	125.0	13.6	18.2
Strontium salicylate	$(C_6H_4OHCOO)_2Sr+2H_2O$	5.31	5.5	2.06	1.5
Stearic acid	$C_{17}H_{35}COOH$	0.03+	(c)	4.33	6.0
Tartaric acid	$C_2H_2(OH)_2(COOH)_2$	137.5	140.8	37.4	60.0
Antimony potassium tartrate	$C_2H_2(OH)_2(COOK)(COOSbO)+\frac{1}{2}H_2O$.	8.52	6.45	Trace.	(c)
Potassium bitartrate	$C_2H_2(OH)_2(COOH)(COOK)$	0.654	0.5	0.014	(h)
Potassium sodium tartrate	$C_2H_2(OH)_2(COONa)(COOK)+4H_2O$.	114.2	83.3	Trace.	(f)
Trichloracetic acid	CCl_3COOH	1,201.0	(d)	Decomp.	(d)
Zinc valerate	$(C_4H_9COO)_2Zn+2H_2O$	1.46	2.0	5.82	2.8

[a] Solid phase anhydrous.
[b] Readily soluble.
[c] Insoluble.
[d] Very soluble.
[e] Sparingly soluble.
[f] Almost insoluble.
[g] Slightly soluble.
[h] Very sparingly.

GENERAL CONCLUSIONS.

During the course of the preceding investigations many points of especial interest have arisen, and since it appears desirable to emphasize some of them more particularly they are briefly summarized here at the end of the bulletin.

1. In order to show the differences between the solubility values as found in the present investigations and those quoted by the pharmacopœia, a comparative table, No. XLV, has been made. The pharmacopœial values in the table are in all cases the reciprocals of the

figures quoted by the U. S. Pharmacopœia in terms of parts of solvent to dissolve one part of the compound. It is of course not certain just what is meant by "part" in the Pharmacopœia, whether weight or volume, and therefore the figures can not be accurately interpreted. An examination of the table shows that of the 35 compounds included satisfactory agreement exists only in the cases of benzoic, camphoric, gallic, and tartaric acids in the aqueous solutions, and of benzoic acid, ammonium benzoate, ammonium salicylate, salicylic acid, and phenol salicylate in the alcohol solutions. Of the remaining results the differences vary from about 5 to 100 per cent.

2. A number of experiments have shown that with the class of compounds dealt with in the present bulletin an apparent impurity which in some cases may be comparatively large does not materially affect the solubility results obtained. It would therefore appear that in many cases solubility determinations are ineffectual as tests for purity. In view of the far more expedient and trustworthy chemical tests, their value as tests for identity is of importance only in certain exceptional cases, consequently the suggestion that has frequently been made that a standard method for solubility determinations be included in the U. S. Pharmacopœia does not deserve much consideration. A brief statement embodying the essential requirements of accurate solubility determinations (intended as physical constants), such as proper temperature regulation, agitation to complete saturation, purity of materials employed, and methods of analysis of solutions, would no doubt be sufficient for all the needs of the pharmacopœia.

3. The solubility curves shown in the accompanying plates present almost every variety of form, some descend or ascend regularly with increase of alcohol, others show maximum or minimum points, while in one or two cases both a maximum and minimum point are present. It would therefore appear that in no case is it possible to predict from the solubility of the substance in alcohol and water separately what it will be in any mixture of these two solvents.

4. It is pointed out that certain advantages are to be gained by pharmacists in adopting the percentage or unit of solvent basis for stating solubility results in place of the antiquated method according to which the amount of solvent required per unit of dissolved substance is given.

5. Attention is called to the fact that the present pharmacopœial purity requirement for certain of the compounds, viz, lead acetate, zinc acetate, sodium benzoate, and lithium salicylate (? ½ Mol. H_2O) is too rigid in view of their unstable character or of difficulty in their purification; whereas in other cases, viz, ammonium benzoate, phenyl salicylate, strontium salicylate, and trichloracetic acid, the

present requirements for purity might be raised without either working an undue hardship upon the producer or increasing the cost to the consumer.

6. The pharmacopœial method for the assay of many of the salts of the organic acids depending upon their incineration and titration of the resulting alkaline residue requires a modification for accurate results. This consists simply in leaching the residue after the first ignition, igniting the unburned carbon and adding the solution of this to the first leachings before making the titration.

7. Quantitative methods of analysis or improvements upon the pharmacopœial processes are suggested for the following salts: Ammonium benzoate, salicylate and valerate, lead acetate, zinc acetate, phenolsulphonate and valerate, sodium phenolsulphonate, phenyl salicylate, potassium bitartrate, and potassium and sodium tartrate.

8. The pharmacopœial statements in regard to the reaction of a number of the salts of the present series toward indicators should be revised.

9. Certain peculiarities in the solubility behavior were noted (*a*) with citric acid which shows two nearly parallel curves for the hydrated and anhydrous forms, (*b*) with potassium citrate, which belongs to that class of compounds which have the power of dividing aqueous alcohol solutions into layers, (*c*) with oleic acid, which presents a striking case of apparently unstable solubility equilibrium at certain concentrations, and finally (*d*) of trichloracetic acid, which unites with alcohol to form the ester in alcoholic solutions of various concentrations.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

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TREASURY DEPARTMENT

Public Health and Marine-Hospital Service of the United States

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HYGIENIC LABORATORY.—BULLETIN No. 68

JUNE, 1910

THE BLEACHING OF FLOUR AND THE EFFECT OF NITRITES ON CERTAIN MEDICINAL SUBSTANCES

By

WORTH HALE

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THE BLEACHING OF FLOUR AND THE EFFECT OF NITRITES ON CERTAIN MEDICINAL SUBSTANCES.[a]

By Worth Hale,

Assistant Pharmacologist, Division of Pharmacology, Hygienic Laboratory, United States Public Health and Marine-Hospital Service, Washington, D. C.

In the early milling of wheat the flour was produced by grinding the wheat between stones, after which it was stored for a varying length of time for the purpose of aging it. This aging process was believed to mature the gluten in some way so that its bread-making qualities were enhanced, but in addition the flour lost during this process a part of its natural yellow color. The resulting flour nevertheless still retained considerable coloring matter, and it was only with the introduction of the roller-milling process that a much whiter flour was produced. At first such flour was objected to on the grounds that although whiter it would not make so good bread as flour ground by the older process, and it is natural, therefore, to find that a yellow tint was considered desirable.[b] But owing to the general introduction of the improved roller process the public opinion changed gradually, so that Kick[c] wrote in a supplement to his book on Flour Manufacture that "now the flour dealers demand the whitest possible flour for their trade." For many years thereafter storing was resorted to to a greater or less extent in order that such a naturally bleached flour could be provided to meet the public demand. But the method was more or less imperfect, in that it not only required large, light, and airy storage warehouses, but also from the fact that large amounts of money were tied up in the flour during the aging period.

It was to hasten and make more complete this natural aging that gave rise to the artificial bleaching of flour. The first patent covering such a process was issued to Beans[d] in 1879, but modern bleaching may be regarded as dating from 1898, in which year a patent was issued to Frichot,[e] a Frenchman, and the great gain in time and

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[a] Manuscript submitted for publication March 12, 1910.

[b] Kick: Flour Manufacture, 1888, p. 250.

[c] Kick: Flour Manufacture, Suppl., 1883, p. 53.

[d] Beans: British Patent No. 2502, 1879.

[e] Frichot: French Patent No. 277751, 1898.

lessened expense of storage to the millers which result led in a comparatively short time to the almost universal adoption of artificial in lieu of the storage system of bleaching formerly practiced. The general spread of artificial bleaching in this country, however, dates from the issuance of the Alsop[a] patent in 1904.

Frichot believed that the ozone which was produced in applying his method was the active bleaching agent, but it has since been shown that ozone does not bleach flour,[b] or at least only to a minor degree, and it was left for two Englishmen to show that the active bleaching substance or substances were certain oxides of nitrogen.[c] Several methods have since been proposed and at the present time one or two general methods are made use of for applying these nitrogen oxides to flour; namely, the electrical or the Alsop process and the chemical. In the Alsop or electrical process a flaming electric arc in the presence of air forms nitrogen peroxide, and this reagent, mixed with an excess of air, is then forced into agitators, where it comes into intimate contact with the flour. In the chemical process nitrosyl chloride is used. This is a gas at ordinary pressure and temperature, but may be liquefied when subjected to about 20 pounds of pressure to the square inch, thus making its shipment, storage, and application to the flour very convenient.

Flour bleached for the trade by one of these processes does not seem to differ in general physical properties, except in being whiter, from an unbleached or naturally aged flour.

It has been shown, however, that flour may be overtreated, and in such a case there are marked changes in physical properties. Such a flour again assumes a yellow hue, depending upon the degree of overtreating, and has a very noticeable musty odor, while the expansion and baking properties of the flour are materially decreased. The most important and characteristic change in commercial flours is that they respond to tests for nitrites, although the amount of nitrite-reacting nitrogen is small, it being generally conceded that the average amount in properly bleached flour approximates only 1 part per million.[d] However, it seems almost certain that until recently the amount present was much greater than this, as Fleurent,[e] a French investigator, reported 20 to 50 parts per million. Ladd,[f] a year later, reported a commercial North Dakota flour with 13 parts per million, and Alway, after examining a large number of Nebraska flours, reported as much as 27 parts, but an average of 6.3 parts, nitrites per million.

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[a] Alsop: U. S. Patent No. 759651, 1904.

[b] Fleurent: Compt. Rend. de la Acad. des Sci., 1906, 142, 180.

[c] The chemistry of the bleaching of flour is very imperfectly understood. It is claimed (Elliot, brief presented in defense of the bleaching of flour to Secretary Wilson and the board of food and drug inspection) that it is merely an oxidation change—but it seems probable that some other action is involved on account of the slight action of oxidizing agents in general.

[d] Wesener and Teller: Jour. of Indust. and Eng. Chem., 1909, I, 700.

[e] Fleurent: Northwestern Miller 1905, 64, 653.

[f] Ladd: Special Bull. No. 9, N. Dak. Exper. Sta., p. 3.

Whether other changes not so clearly demonstrable occurred in commercially bleached flour aside from the decrease in color and the presence of nitrite-reacting material has been made the subject of a number of investigations. These have been very contradictory. On the one hand certain observers have claimed that the acidity was increased; that the bread-making qualities were lessened; that the digestibility of the glutens and starches were lessened; and, finally, that such flours contained products with a markedly toxic effect upon animals. On the other hand, all of these contentions have been denied by other investigators, so that the present status of the whole question of bleaching flour, and especially with regard to its effect upon the public health, is far from determined. It was in view of these contradictions that the experiments reported in the following pages were undertaken.

Experiments were carried out along two distinct lines. In the first place, a series of experiments were taken up to determine whether any changes occurred in bleached flour which would alter its nutritive value, especially as determined by its comparative digestibility, and whether or not such flour contained toxic products formed by the action of the bleaching agent upon one of the constituents of the flour. Experiments were also carried out to determine what effect sodium nitrite would exert upon the action of certain digestive ferments, and to what extent small amounts would alter the toxicity of other drugs.

The flour used in by far the greater number of experiments recorded below was made from hard wheat and labeled "Fancy Patent, guaranteed natural color, not chemically bleached." A test for nitrites was made, however, but gave negative results.

For the purpose of bleaching portions of this flour in small lots nitric oxide gas was generated in a nitrometer as follows: Double normal solutions of sodium nitrite, potassium iodide, and sulphuric acid were made up with distilled water; 5 c. c. of the sodium nitrite solution were then placed in the receiving cup and allowed to replace the mercury with which the nitrometer was filled in order to displace the air; 10 c. c. of the potassium iodide solution were then added in the same manner, thus washing in most of the sodium nitrite; 5 c. c. sulphuric acid solution were then added which, reacting with the nitrite and the potassium iodide solution, liberated nitrous and hydriodic acids according to the following equations:

$$2NaNO_2+H_2SO_4=Na_2SO_4+2HNO_2$$
$$2KI+H_2SO_4=K_2SO_4+2HI$$

The hydriodic and the nitrous acid then reacted according to the following equation:

$$2HNO_2+2HI=2H_2O+2I+2NO.$$

The nitric oxide thus formed is changed in the presence of air into nitrogen peroxide, and hence, when mixed with flour in the presence of air in excess, it exerts its bleaching action through the formation of this substance.

The method used in the laboratory bleaching of flour as detailed in the experiments which follow is essentially the same as that suggested by Alway.[a] A slight modification, however, was adopted. A large flask is fitted with a cork notched at the sides, through which is inserted a glass tube ending in a capillary point. This is then attached by a long piece of rubber tubing with the nitrometer. A weighed amount of flour having been placed in the flask, which should have a capacity of three or four times the weight of flour used, and the air in the connecting rubber tubing and glass tube having been replaced by nitric oxide gas from the nitrometer, the cork is inserted tightly and the desired amount of gas is slowly relieved. During the delivery the flask containing the flour is vigorously shaken to keep the flour in constant agitation, thus obviating an overtreatment of the flour at the top of the flask, as might be the case were the gas introduced without shaking.

As different flours require varying amounts of gas to obtain the best bleaching effect, a number of experiments were made to determine approximately the amount which would result as determined by Pekar's method[b] in the greatest bleaching to the flour under consideration. Lots of 200 to 1,000 grams each were placed in a 1-liter flask and gas varying in amounts from 10 c. c. to 1,000 c. c. per kilo were added. The results were as follows:

Bleaching effect of varying amounts of nitric oxide gas upon the same flour (Fancy patent).

Series.	Cubic centimeters of gas per kilogram of flour.	Nitrite N per million.	Color.	Odor.
1	0	0.0	Yellow	Natural.
2	10	1.4	Light yellow	Do.
3	20	1.6	do	Do.
4	30	2.1	White	Do.
5	50	7.2	Whitish	Slight, not unpleasant.
6	100	8	do	Greater change, not unpleasant.
7	200	5.6	Yellow	Musty odor.
8	1,000	5.2	Deep yellow	Do.

It will be seen from the above table that the flour bleached with approximately 30 c. c. of gas gave the best results, giving the whitest flour without destroying the natural odor or if at all to a very slight degree.

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[a] Alway: Bull. 102 Agr. Exper. Sta., Nebraska, The effect of bleaching upon the quality of wheat flour, p. 31.

[b] Kick: Flour Manufacture 1888, 251.

Three samples of flour were accordingly bleached with the object of obtaining a slightly overtreated flour, one considerably overtreated and one markedly overtreated. For the first, 3 kilograms of flour were divided into lots of 1 kilogram each and bleached with 50 c. c. nitric oxide gas per kilogram; for the second, 4 kilograms of flour divided into lots of 1 kilogram each were bleached with 100 c. c. nitric oxide gas per kilogram; for the third, 4 kilograms, divided into lots of 1 kilogram each, were bleached with 1,000 c. c. nitric oxide gas per kilogram.[a] These flours of each series were then thoroughly mixed and tested for nitrites. That bleached with 50 c. c. gas gave a test for 6.1 parts of nitrogen as nitrite per million, that with 100 c. c. gas, 9.2 parts per million, and the third lot, bleached with 1,000 c. c. gas, 6.3 parts per million.

Having thus secured a suitable amount of flour, bleached to varying degrees, the following experiments were carried out:

DIGESTION EXPERIMENTS.

Flour gluten.—A number of investigators have carried on work to determine what effect artificial bleaching would have on the digestibility of flour and bread. Ladd[b] tested the action of the salivary, peptic, and pancreatic ferments on starch and gluten of bleached and unbleached flour in both the raw and the cooked state and concluded that bleaching delayed the digestive process. Halliburton[c] also carried on a somewhat extensive investigation concerning the action of salivary and of peptic enzymes on the starch and gluten of commercially bleached and unbleached flour. In addition, he also investigated the effect of adding nitrites to starch solutions and to fibrin, thus testing the salivary and peptic digestion in the presence of nitrites. His conclusions were that the bleaching process and also the simple addition of nitrites invariably delayed the digestion process. Snyder,[d] however, in a large number of tests of digestibility upon man and with artificial gastric juice was unable to detect any differences whatever in the rate of digestion between bleached and unbleached flour or between bread baked from such flours.

Wesener and Teller[e] also were unable to note any difference in the degree of peptic digestion.

These variable results are difficult to explain and suggested the desirability of further tests along similar lines in an effort to determine whether bleaching or the artificial addition of nitrites as such in small amounts would effect the rate of digestion.

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[a] A Mariotte bottle with a capacity of 1,000 c. c. was fitted up and the gas manufactured in the same manner as for the smaller amounts.

[b] Ladd: Chemical News, 1909, 99, 127.

[c] Halliburton: Journ. of Hygiene, 1909, IX, 170.

[d] Snyder: Bulletin 111, Flour Bleaching, Minn. Agri. Exp. Sta., 1908, pp. 124–126.

[e] Wesener and Teller: Jour. of Indust. and Eng. Chem., 1909, I, 709.

Owing to the adhesive, intractable character of the gluten of flour, the investigation of its digestibility is beset with many difficulties. The method used by Halliburton was gravimetric; that is, of carrying on digestion of weighed amounts and then estimating the amount of insoluble residue. Snyder also estimated the insoluble residue determining the per cent of nitrogen which remained after a certain time. Ladd determined the length of time necessary to completely digest the gluten from 10 grams of flour. This was placed on glass wool in test tubes with perforated bottoms to allow ingress of the digestive agent. This method, after a trial of Halliburton's filtration method, was adopted for the following experiments.

Flour, from the same lot, bleached according to the method already described, was used. Three lots of 10 grams each were weighed out and made into a dough using 5.5 c. c. of distilled water. After standing for one hour these were washed in cold tap water until the starch was entirely removed. Each lot of the gluten thus obtained was then massed into a *compact* ball and placed on top of glass wool in perforated tubes. Care was taken that the holes in the bottom of the tubes were of equal size, so that the diffusion of digestive fluid would be the same in each case. The tubes were then placed in beakers of the same size and to each beaker 150 c. c. of an artificial gastric juice[a] was added. The beakers were then placed in an incubator and fifteen hours later regular hourly and, when indicated, half hourly examinations were made until the mass of gluten was digested. The results of several series of experiments were as follows:

TABLE I. *Determination of the rate of digestion of unbleached and bleached flour glutens in artificial gastric juice.*

Series.	Flour.	Temperature of oven.	Time till complete digestion.
			H. m.
I....	Unbleached....	38	24 05
	50 c. c. bleached..	38	26 00
	100 c. c. bleached..	38	27 00
II	Unbleached....	38	23 30
	50 c. c. bleached..	38	26 00
	100 c. c. bleached...	38	27 00
III...	Unbleached....	38	23 00
	50 c. c. bleached...	38	24 30
	100 c. c. bleached..	38	25 00
IV..	Unbleached....	40	21 50
	50 c. c. bleached...	40	25 40
	100 c. c. bleached...	40	26 30
V	Unbleached....	40	21 30
	50 c. c. bleached	40	23 30
VI.....	Unbleached....	42	19 00
	50 c. c. bleached...	42	19 50
	100 c. c. bleached...	42	21 50

[a] The artificial gastric juice was made up using 1 gram of scale pepsin to 1,000 c. c. of 0.25 per cent hydrochloric acid.

Somewhat later a further series of experiments were carried out in the manner described above, excepting that flours bleached with smaller amounts of nitric oxide were used. The results are given in Table II.

TABLE II.—*Determination of the rate of digestion of unbleached and bleached flour gluten in artificial gastric juice.*

Series.	Flour.	Temperature.	Time till complete digestion.
		°	*H. m.*
I	Unbleached	40	19 30
	10 c. c. bleached	40	18 00
	20 c. c. bleached	40	20 00
	30 c. c. bleached	40	18 00
II	Unbleached	40	20 00
	10 c. c. bleached	40	19 00
	20 c. c. bleached	40	21 30
	30 c. c. bleached	40	22 00
III	Unbleached	40	19 30
	10 c. c. bleached	40	20 30
	20 c. c. bleached	40	20 00
	30 c. c. bleached	40	20 30
IV	Unbleached	40	20 00
	10 c. c. bleached	40	22 30
	20 c. c. bleached	40	21 30
	30 c. c. bleached	40	22 00

Two samples of commercially bleached flour were also tested, using the above method and using the same flour as in the controls in Table I as a control in this series. As the flours were all from different grists the amount of gluten in each was estimated and then amounts yielding approximately the same weight of gluten as the unbleached control were taken for the tests. The controls were digested after an average of 20 hours and 30 minutes; commercially bleached sample No. 140 after an average of 18 hours and 50 minutes, or somewhat more rapidly than the unbleached sample; commercially bleached sample No. 11211, after an average of 23 hours and 20 minutes, or somewhat more slowly than the control.

Summing up the results of the whole series of experiments it may be concluded that for flours of the same grist those bleached with nitric oxide gas are somewhat more slowly digested than the unbleached control. With the smaller amounts of nitric oxide gas used in the bleaching process there is also a closer approach to the same rate of digestion as is exhibited by the control. In no case also could such marked delay in rate be demonstrated as reported by Ladd, although the experimental procedure was made as nearly parallel as possible. The results of these experiments, however, agree quite closely with those reported by Halliburton so far as the degree of lessened digestion is concerned.

In the case of flours of different grists, as in the commercial samples, no explanation appears for the variable results obtained. It suggests, however, that comparisons between the digestibility of glutens of wheats derived from different sources may be misleading, owing to the possible variability in the character of the glutens.

Egg albumen and nitrites.—In addition to his work on the digestibility of flour, Halliburton[a] also investigated what effect the addition of nitrites to fibrin and to an artificial gastric juice would have upon the rate of digestion of the fibrin. In all cases he reported that such addition, even when only in 1 part to 32,000, markedly lessened the rate of digestion.

This work of Halliburton suggested a similar line of investigation, excepting that greater dilution of nitrite was used and that coagulated egg albumen was used in place of fibrin. The method employed was that of Mett, in which glass tubing of small bore is filled with egg albumen and then heated (for five minutes in boiling water) until thoroughly coagulated. The tubing is then broken into pieces of about 2 cm. length and placed in the artificial digestive fluid. The rate of digestion is then determined by noting after a stated interval the distance from the ends of the tube that digestion has progressed. The measurements were made with a micrometer of special design,[b] reading to 0.005 mm.

The artificial gastric juice used in these experiments was made up according to the formula already given (p. 10). Aside from the controls, to which no nitrite was added, the nitrite was mixed directly with the digestive fluid.

From five to ten tubes were used in each test, and the average from the ten to twenty readings thus taken (the figures representing 0.005 mm.) was used as a basis of comparison. The series of tubes were placed in beakers of the same size and subjected to the action of 50 c. c. of the digestive fluid to which sodium nitrite had been added in varying dilution. The oven temperature was maintained at 38° to 40° and the readings made at varying intervals, as noted in the tables which follow. Only averages are recorded. (See Table III.)

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[a] Halliburton: Journ. of Hygiene, 1909, IX, 170.

[b] Schultz: Adrenalin and Adrenalin-like bodies, Bull. 55 U. S. P. H. and M. H. S., Washington, p. 50.

Table III.—*Determination of the rate of digestion of egg tubes in artificial gastric juice as affected by the addition of sodium nitrite.*

Series.	Number of readings.	Amount of sodium nitrite.	Hours.	Total length[a] of digestion.	Per cent (control = 100 per cent).
I[b]	12	None.	24	6,940	100.0
	12	1- 5,000	24	4,980	71.8
	12	1- 20,000	24	6,275	90.4
II	10	None.	18	8,155	100.0
	10	1- 20,000	18	6,870	84.2
	10	1- 40,000	18	7,955	97.6
III	20	None.	18	12,030	100.0
	20	1-100,000	18	11,340	94.3
IV	20	None.	18	14,580	100.0
	20	1-200,000	18	13,650	93.6
V	20	None.	18	16,115	100.0
	20	1-200,000	18	15,965	99.1
VI	18	None.	18	14,605	100.0
	18	1-200,000	18	14,400	98.6
VII	10	None.	18	9,580	100.0
	10	1-200,000	18	9,220	96.3

[a] Factor=0.005 gives readings in millimeters.
[b] Acidity 0.1 per cent. In all other cases 0.25 per cent.

To facilitate comparison, the following summary of the averages in Table III is given:

SUMMARY.

Number of readings.	Amount of sodium nitrite.	Per cent (control=100 per cent).
110	None.	100.0
12	1- 5,000	71.8
22	1- 20,000	87.3
10	1- 40,000	97.6
20	1-100,000	94.3
68	1-200,000	96.9

From the summary it is readily seen that the addition of small amounts of nitrites retards the digestion of egg albumen to a considerable degree, even when the dilution is approximately 1 part of nitrogen as nitrite per million (1:200,000 sodium nitrite); markedly when larger amounts are used.

EXPERIMENTS ON ANIMALS.

Having thus determined the comparative digestibility of bleached and unbleached flour a large number of toxicity experiments were carried out on animals, using mice, rats, and rabbits. The earlier experiments consisted in testing the effect of bread and flour on nutrition, the later ones the toxic effect of extracts of flour and of the nitrites combined with other drugs.

Experiments with bread.—Although these experiments were very incomplete, they are to be reported here on account of the rather unexpected results which were obtained. Thirty mice from the same lot were divided into lots of 10 each and weighed, each lot weighing 135 grams, or an average of 13.5 for each mouse. They were then placed in cages of the same size and pattern which were located in a large animal room, thus insuring like conditions as to temperature, light conditions, etc. Each lot was then fed an average of 5 grams of bread per mouse as a daily ration.

The bread was made up from unbleached flour and from two bleached flours, one bleached with 100 c. c. and one with 1,000 c. c. nitric oxide gas. The three lots of flour were all made into bread under as nearly as possible the same conditions, using the same amounts of yeast, salt, keeping the dough at the same temperature, and baking the three lots at the same temperature for the same length of time.

The bleached flours were both evidently overtreated, as one contained 9.7 and the other 6.3 parts nitrogen as nitrites per million, and both had a musty odor, which was most pronounced in the case of that bleached with 1,000 c. c. gas. Only slight differences in bread-making qualities were noticeable in the case of the 100 c. c. gas flour, the dough mass appearing somewhat more "sticky" with equal amounts of water than the unbleached control, but the raising qualities did not seem to be materially affected. In the case of the 1,000 c. c. gas bleached flour, however, the dough mass was markedly more sticky and the yeast action was almost nil.

The results of feeding such bread are given in the following table:

TABLE IV.—*Bread feeding; unbleached and bleached flour bread fed to white mice; daily ration, 5 grams.*

Date.	Average weight of mice.			Number mice living.		
	Un-bleached.	Bleached. 100.	Bleached. 1,000.	Un-bleached.	Bleached. 100.	Bleached. 1,000.
1909.	*Grams.*	*Grams.*	*Grams.*			
Dec. 7	13.50	13.50	13.50	10	10	10
9	13.80	13.80	13.80	10	10	10
11	14.20	13.60	13.90	10	10	10
13	13.70	13.70	13.90	10	10	10
15	13.80	13.40	13.80	10	10	10
17	13.40	13.40	13.80	10	10	10
20	13.50	12.80	13.40	8	10	9
22	13.40	12.30	12.90	8	10	9
24	12.75	11.50	13.10	8	10	8
27	11.25	10.60	12.10	4	6	8
29				3	3	7
30				1		7
31		10.15		0	1	7

From this table it will be noted that the markedly overtreated flour acted least deleteriously, while the unbleached and that bleached with 100 c. c. gas were much more so. Only the one series was fed on bread in this way, and it is not possible to say whether such a result could invariably be obtained.

A further series of mice were fed in a somewhat comparable way, however, but in this case upon unleavened dough cakes. The mice were divided into three lots of five each, the total weight of which was approximately the same for each lot. Each mouse was placed in separate mouse jars and fed on cakes made up from the various flours used in the bread-feeding experiments. The cakes were made by making up a stiff dough by adding to the flour one-half its weight of a 1 per cent salt solution and dried at 60°. The feeding was continued for approximately the same period as in the experiments with bread. At the end of this time only one mouse was dead, but subsequently others also died. The following table gives the loss in weight during twenty-two days:

TABLE V.—*Determination of nutritional changes in white mice fed upon dough cakes made from unbleached and bleached flour.*

	Loss in weight.		
	Unbleached.	Bleached 100 c. c. gas.	Bleached 1,000 c. c. gas.
Mouse No. 1	[a] 2.34	2.64	2.14
2	5.06	5.96	1.85
3	3.21	1.86	2.87
4	2.01	2.68	3.59
5	3.30	1.71	1.32
Total original weight	75.63	74.82	79.71
Total loss in weight	15.92	14.85	11.77
Per cent loss in weight	21.20	19.80	14.60

[a] Died after 12 days.

From a review of this table it will be noted that again the unbleached flour seemed to exert the most marked, while the most overtreated the least deleterious action. This unexpected result was further shown by the fact that the mice receiving the most overtreated flour were all alive at the end of thirty days, while two in each of the other lots had died probably from malnutrition. No explanation of these results can be suggested.

Alcoholic extracts.—Further animal tests were also undertaken along lines similar to those originally suggested by Ladd, who made alcoholic and aqueous extracts of overtreated, commercial, and unbleached flours. These were fed to rabbits in his experiments by means of a stomach tube, and both the overtreated and the commercial flours were found to be quite markedly toxic, the extract from about 250 grams of flour being sufficient to kill rabbits after from fifteen minutes to three and one-half hours. With the unbleached extracts, however, which were fed in the same way, no symptoms other than some discomfort resulted, while the introduction of like quantities of 16 per cent alcohol also was devoid of any apparent toxic action.

In the reports of these experiments unfortunately the author does not give a detailed account of the various procedures used in making these extracts, and this may account for the failure of the investigators who have tried to duplicate Ladd's results. Wesener and Teller[a] carried out experiments of a similar nature, using an unbleached flour, one containing 2.8 parts of nitric nitrogen, and one containing 30 parts per million. These flours were extracted with 90 per cent alcohol and then evaporated at a low temperature in vacuo. Feeding such extracts to rabbits gave negative results so far as any apparent toxic symptoms were concerned, and post-mortem examinations were also negative. Luff and Wilcox,[b] in England, also using extracts of bleached flour, reported negative results. Others in America (private communication) have also failed to obtain toxic results, but in view of the fact that negative results are less certain than positive findings it was thought worth while to repeat the experiments, following Ladd's methods as closely as possible.

For this purpose several lots of flour were taken from an unbleached lot and bleached with varying amounts of nitric oxide gas in the manner already described. Four lots were taken, one unbleached to serve as a control, one bleached with 50 c. c. nitric oxide gas, one bleached with 100 c. c., and one with 1,000 c. c. The flour bleached with 50 c. c. gas and containing 6.1 parts nitrite nitrogen per million did not appear to be overtreated as judged by its color and odor. The lot bleached with 100 c. c. and containing 9.2 parts nitrite nitrogen per million was evidently somewhat overtreated, having a somewhat musty odor. The lot bleached with 1,000 c. c. gas containing 6.3 parts per million nitrite nitrogen was evidently very much overtreated, being of a fairly deep yellow color and having a pronounced musty odor. One thousand grams each of these flours were taken, divided into lots of 500 grams each, and placed in carefully cleaned Florence flasks of 2,000 c. c. capacity, together with 900 c. c. of 95 per cent alcohol, or 1,800 c. c. of alcohol for each 1,000 grams of flour taken. The flasks were then well shaken several times each day during a period of seven days. Then, after thorough shaking, the flour and alcoholic extract were poured into a Buchner filter, the filtration being hastened by use of a vacuum pump. As the filtrate thus obtained contained considerable flour, it was again filtered through an ordinary filter until clear. About 940 c. c. of a yellowish solution was obtained from each lot of flour with the exception of that bleached with 1,000 c. c gas, which, on account of its marked sticky consistency, gave only 770 c. c.

To reduce the volume of alcohol these filtrates were evaporated in vacuo (60 to 75 mm. mercury) in an atmosphere of hydrogen at a temperature of 25° to 30°. At the very last of the distillation the temperature rose, but in no case to more than 41°. The hydrogen gas was generated in as nearly as possible the same way for each distillation, the gas being generated by the action of dilute sulphuric acid upon zinc, and was introduced into the flask containing the alcoholic flour extract through a very fine capillary. The evaporation in each case was continued until the alcohol was almost completely driven off. The oily residue was then taken up with small amounts of 50 c. c. alcohol until the flask had been entirely washed out, care being taken, however, to bring up the total volume of oily extractive and alcohol to exactly 100 c. c., thus giving an extract 1 c. c. of which equaled 10 grams of flour.

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[a] Wesener and Teller: Jour. Indust. and Eng. Chem., 1909, I, 700.

[b] Luff and Wilcox: From Halliburton, Jour. of Hygiene, 1909, IX, 175.

Each extract thus obtained was then tested by the removal of 1 c. c. portions for acidity, using phenolphthalein as an indicator, with the following results:

Sample.	Total acidity as HCl.	Color.	Odor.
Unbleached	0.054 per cent	Yellow	
50 c. c. gas	0.097 per cent	do	
100 c. c. gas	0.108 per cent	Pale yellow	Acid.
1,000 c. c. gas	0.468 per cent	Deep yellow	Do.

EFFECT OF EXTRACTS GIVEN SUBCUTANEOUSLY.

The animals used in this series of experiments were white mice and rats. These were brought to the operating room in small lots on the day they were to be used, carefully weighed and put into separate jars. Until brought to the operating room they were kept together (rats and mice in separate cages) in large cages and hence under approximately the same conditions as to food, temperature, etc. (These factors are of considerable importance in order to secure animals which will react fairly uniformly to experimental procedures.)

Since mice are easily affected by alcohol in small doses the 50 per cent alcoholic extracts were evaporated in shallow glass evaporating dishes each dish containing a single dose of 1 to 1.5 c. c., an amount obtained from the extraction of 10 to 15 grams of flour. Having continued the evaporation (slowly, at room temperature) until a gummy, oily residue remained, this was taken up with a small amount of distilled water. As the oily residue did not mix with water easily it was emulsified as much as possible by repeatedly drawing it up into a syringe and then forcing it out again. It is of interest to note also in this connection that the bleached extracts were more easily emulsified than the unbleached and those from the 1,000 c. c. and 100 c. c. flour more easily in the order named.

The details of the following experiment afford the only instance where toxic effects followed the injection of the unbleached flour extract.

December 28, 1909. 1 c. c. unbleached flour extract. Evaporated to gummy residue; added 0.5 c. c. distilled water. Not easily emulsified. Mouse, 24.71 grams weight; 1.25 p. m., injected beneath skin of back; 1.35, apparently depressed, quiet, lies stretched out; 2.15, normal, no further symptoms; December 29, 1909, alive.

Four other mice were injected in like manner with amounts varying from 1 to 1.5 c. c. (the extract from 10 to 15 grams flour) but none of these showed any effects as a result of the injection. The weights of the mice, dose used, and results are summarized in the following table:

TABLE VI.—*Effect of an alcoholic extract unbleached flour on white mice when given subcutaneously, alcohol evaporated.*

Date.	Weight.	Dose.[a]	Results.
1909.	*Grams.*	*C.c.*	
Dec. 28	23.63	1.0	No symptoms.
28	24.71	1.0	Slightly sick.
30	14.50	1.0	No symptoms.
30	13.50	1.5	Do.
30	14.32	1.5	Do.

[a] 1 c. c. extract equals extractive from 10 grams flour.

Rats were also injected subcutaneously with the above unbleached flour extract, but showed no effects from the injections. The results are given in the following table:

TABLE VII.—*Effect of alcoholic extracts of unbleached flour on white rats when given subcutaneously; alcohol evaporated.*

Date.	Weight of rat.	Dose.	Result.
1910.	*Grams.*	*C. c.*	
Jan. 4	132	8.0	No symptoms.
4	189	8.0	Do.
4	125	8.0	Do.
4	195	8.0	Do.

Fifty cubic centimeters nitric oxide bleached flour extract.—In like manner mice from the same lot were injected with the extract obtained from flour bleached with 50 c. c. nitric oxide gas. In the series four mice, varying in weight from 16.44 to 22.90 grams, were injected with 1.5 c. c. doses (15 grams of flour). None of the mice died, but all were made somewhat ill; the following protocol illustrating the effect produced:

January 11, 1910, mouse, 17.06 grams weight; 1.5 c. c. gas extract allowed to evaporate at room temperature to gummy residue; emulsified with 5 c. c. distilled water—does not emulsify well; 10.55 a. m. injected beneath skin of back; 11.30 not very sick, somewhat drowsy; 1.30 somewhat restless; at 2.30 changes position frequently as if restless—sudden starts, eyes kept closed; January 14, 1910, apparently well.

A summary of these experiments is to be found in the following table:

TABLE VIII.—*Effect of an alcoholic extract of flour bleached with 50 c. c. nitric oxide gas on white mice, subcutaneous injection; alcohol evaporated.*

Date.	Weight.	Dose.	Results.
1910.	*Grams.*	*C. c.*	
Jan. 11	16.44	1.5	Only slightly sick.
11	17.06	1.5	Sick.
14	22.90	1.5	Slightly sick.
14	20.98	1.5	Quite sick.

One hundred cubic centimeters nitric oxide bleached flour.—A series of mice were also injected with varying amounts of the extract prepared from the flour bleached with 100 c. c. nitric oxide gas. This extract proved to be materially more toxic, so that of the 10 mice injected 6 died and 4 lived. The doses varied from 0.75 c. c. to 1.5 c. c. The following protocol will serve to indicate the general character of the symptoms, but in certain instances, although death resulted, the symptoms were less marked:

December 29, 1909; 1 c. c. alcohol extract, 100 c. c. gas evaporated at room temperature to gummy residue, taken up and emulsified with 0.5 c. c. distilled water; mouse, 17.46 grams weight; 11.25 injected beneath skin of back; 11.30, respiration difficult, panting, very quiet, fur mussed; 11.40, respiratory distress marked; 12.10, crawls about cage, but does not raise body, hind legs scarcely used at all; 12.20, more active, but still drags body; 2.20, able to drag up hind feet, does not drag body quite so much, head and anterior part of body tremble markedly; December 30, 1909, alive, does not show symptoms of sickness aside from keeping eyes shut; active.

The following table summarizes the results obtained:

TABLE IX.—*Determination of toxicity of an alcoholic extract of flour bleached with 100 c. c. nitric oxide gas for white mice, subcutaneous injection; alcohol evaporated.*

[1 c. c. = extract from 10 grams of flour.]

Date.	Weight.	Dose.	Result (— = alive; + = dead).	Time till death.
1909.	*Grams.*			*H. m.*
December 29	17.46	1.0	—	....
29	14.12	0.75	+	12 45
29	13.06	0.75	+	13 40
31	14.10	1.5	+	13 50
31	12.98	1.5	+	1 30
31	11.54	1.5	+	7 15
31	18.73	1.0	—	....
31	18.19	1.0	—	....
31	15.14	1.0	+	14 45
1910.				
January 10	19.10	1.0	—	....

In like manner extracts of flour bleached with 100 c. c. nitric oxide gas were injected subcutaneously into rats. In this series, however, it proved to be less toxic than in the case of the mice experiments. Nevertheless, in every case there were some indications of toxic action and in one case death resulted. Six and 8 c. c. doses were used and after evaporating off the alcohol the gummy residue was made up to 3 c. c. by the addition of distilled water. Upon emulsification this was injected beneath the skin of the back. A summary of the results is given in the following table.

TABLE X.—*Determination of toxicity of an alcoholic extract of flour bleached with 100 c. c. nitric oxide gas for white rats; subcutaneous injection.*

Date.	Weight.	Dose.	Result.
1910.	*Grams.*	*C. c.*	
January 5	125	6.0	Sick; lived.
5	152	6.0	Do.
6	139	8.0	Died, 19 hours.
7	107	8.0	Sick; lived.
7	116	8.0	Do.

One thousand cubic centimeters nitric oxide flour extract.—The alcoholic extract of this flour was very different from the other flours in physical properties, being much more acid, and offering no difficulties to complete emulsification with distilled water.

This extract was given subcutaneously to five mice in varying doses, and death resulted in every instance. The quantities decided upon for injection were placed in evaporating dishes and the alcohol allowed to evaporate spontaneously, after which distilled (0.5 c. c.) water was added. After emulsification this was injected beneath the loose skin of the back. The following protocol is illustrative of the symptoms observed:

December 29, 1909; 0.75 c. c. alcoholic extract evaporated to gum; emulsified with 0.5 c. c. distilled water; mouse, 19.0 grams weight; 1.52, injected subcutaneously, some loss of dose; 2.20, mouse trembles, sick; 3, trembles markedly, sudden starts, circles to one side; found dead 5 a. m., December 30, 1909.

In certain cases no trembling was observed, but rather symptoms of depression, the body being dragged about the cage, the eyes kept shut, and the respiration being labored.

A summary of this series of experiments is given in Table XI.

TABLE XI.—*Determination of the toxic effect of an alcoholic extract of bleached flour (1,000 c. c. nitric oxide gas) upon white mice; subcutaneous injection.*

Date.	Weight.	Dose.	Time till death.
1909.	*Grams.*	*C.c.*	*H. m.*
December 28	24.90	1.0	1 15
28	18.96	0.75	9 45
29	19.00	0.75	11 47
29	20.11	0.75	11 50
30	22.23	1.0	12 30

On account of the high acidity of this extract it was believed that the toxicity might depend upon an acid intoxication. To determine this point a portion of the extract was neutralized with normal sodium hydrate. Having added to the volume of the extract by this procedure somewhat larger doses were injected. As was anticipated, the toxicity was somewhat lessened as is shown by the results given in the following table:

TABLE XII.—*Determination of the toxic effect of a neutralized alcoholic extract of bleached flour (1,000 c. c. nitric oxide gas) upon white mice; subcutaneous injection; alcohol evaporated.*

Date.	Weight.	Dose.	Time till death.
1910.	*Grams.*	*C. c.*	*H. m.*
January 19	14.85	1.5	4 15
19	14.70	1.5	30 40
19	18.51	1.5	Sick, lived.

A series of toxicity experiments were also carried out on white rats, using, however, the unneutralized extract. Five rats were used, the injections being made beneath the skin of the back. The doses, varying from 3.5 to 5 c. c., after evaporating off the alcohol, were each rediluted with 2 c. c. distilled water in order to make the gummy residue suitable for hypodermic injection.

The results are found in the following table:

TABLE XIII.—*Determination of the toxic effect of an alcoholic extract of bleached flour (1,000 c. c. nitric oxide gas) for white rats; subcutaneous injection.*

Date.	Weight.	Dose.	Time till death.
1909.	*Grams.*	*C. c.*	*H. m.*
December 31	91	3.5	12 15
31	129	4.0	21 10
1910.			
January 6	207	5.0	34 00
7	173	5.0	19 10
7	146	5.0	16 35

In summarizing the results of these experiments it may be said that alcoholic extracts of unbleached flour appear to be entirely devoid of toxic action. On the other hand, when alcoholic extracts of bleached flour, made up in as nearly as possible the same way, the toxicity is faintly to markedly developed, the degree being in general proportionate to the amount of nitric oxide gas used in bleaching. Thus, the flour bleached with 50 c. c. of gas extract on injection caused illness only; that bleached with 100 c. c. gas caused illness in part of the animals, but death in by far the greater proportion of cases where mice were used, but exceptionally in the case of rats; that bleached with 1,000 c. c. gas caused death invariably in the case of both mice and rats, although, upon neutralization of the acidity, its toxic action was somewhat lessened.

It has been stated[a] that the toxicity to rabbits in the case of Ladd's experiments may have been due to the presence of hydrochloric acid, zinc chloride, or arsenic in the extracts used, being possibly generated in the process of manufacturing the hydrogen gas.

Unfortunately no analysis of the gas used in my experiments was made, but great care was taken in carrying out the various steps in the process of manufacturing the hydrogen gas used, the zinc and the sulphuric acid being of the same lot for the four distillations, thus insuring rather certainly that the added gas was the same for the several extracts. If hydrochloric acid, zinc chloride, or arsenic were being carried over in one instance, there is at least no reason to believe that these were not generated in all cases alike and approximately to the same degree. That such toxic agents were not introduced in this process seems probable at least for the unbleached extract, because it not only lacked toxic properties, but its acidity in terms of hydrochloric acid was not greater than is normal for flour. It is further emphasized also by the relatively greater acidity of the various extracts in a general ratio to the larger amounts of nitric oxide gas used, a result which would be exceedingly unusual if this acidity were dependent on hydrochloric or any other acid formed in the process of generating hydrogen gas, since, as has been stated, the process was as nearly as possible the same in all instances.

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[a] Elliot: Am. Hay, Flour, and Feed Journal, 1909, XIV, No. 2, p. 28.

It seems safe to conclude, therefore, that the bleached flour used in these experiments contained toxic properties, which were not entirely due to the high acidity caused by bleaching.

EFFECT OF EXTRACTS BY THE STOMACH.

Having shown that the extracts of bleached flour were poisonous to white mice and rats, it was thought worth while to feed them to rabbits to determine whether toxic effects at all comparable to those described by Ladd could be induced. The extracts of flour bleached with 50, 100, and 1,000 c. c. gas and of unbleached flour were used. In all cases 25 c. c. doses (representing 250 grams of flour) were given through a stomach tube. The alcohol was completely evaporated off at room temperature in all cases and distilled water was then added so as to make the total volume up to from 10 to 12 c. c. As has already been described, the emulsification of the unbleached gummy extract was very difficult, that of the 50 c. c. gas flour less, of the 100 c. c. flour still less, and of the 1,000 c. c. gas flour least difficult. The animals were weighed previously to the administration of the extracts, and in no case were toxic symptoms clearly apparent; also subsequently, to determine what changes in weight might result. These results were also practically negative, although in the first series the rabbits receiving the markedly overtreated flours lost the most in weight, but in the second series there was practically no change in weight. These results are recorded in the following table, the change in weight being the maximum change recorded for the six days the animals were under observation:

TABLE XIV.—*Effect of 25 c. c. doses of alcoholic extracts on rabbits; alcohol evaporated; given by the stomach.*

Series.	Amount bleaching agent.	Weight before.	Weight after.	Percentage change in weight.
I	Unbleached	1,162	1,115	4.0 loss.
	50 c. c. gas	1,090	1,100	.9 gain.
	100 c. c. gas	1,132	990	12.5 loss.
	1,000 c. c. gas	1,000	737	26.3 loss.
II	Unbleached	1,379	1,367	.9 loss.
	50 c. c. gas	1,629	1,614	.9 loss.
	100 c. c. gas	1,478	1,450	1.9 loss.
	1,000 c. c. gas	1,555	1,517	2.4 loss.

SODIUM NITRITE, ACTION ON OTHER DRUGS.

In the course of a series of experiments with the coal-tar antipyretics to determine what effect certain other drugs would have upon their toxicity, certain experiments were made using sodium nitrite.

While the amount used in combination with the antipyretic drugs was greatly in excess of that found in bleached flour, it nevertheless had such a pronounced effect that these experiments will be recorded in some detail in this paper, and especially so because they suggested many of the experiments which follow, in which very much smaller amounts of nitrite were used. In this series of experiments white mice of the same lot were used as experimental animals. They were first carefully weighed, placed in separate mouse jars, and then fed upon cakes made up with cracker meal, to which the drug or combination of drugs had been added. Each cake represented 4 grams of the meal (made up by the addition of about an equal amount of water and dried at 60° C.) and constituted the daily ration for each mouse. As the cakes were rarely eaten entirely up, the daily amount of drug ingested varied to some extent, being somewhat less than the amount computed as the daily dose.

Control experiments were always carried out using unmedicated cakes and also cakes in which each of the drugs to be used in combination was incorporated. The first step was to determine the length of time mice kept under such conditions would live on an unmedicated diet as well as upon cakes containing sodium nitrite. As the latter drug was to be added to the drugs under investigation without reducing their doses, it was planned to give sufficiently small amounts that these cakes would be only slightly toxic. Cakes were accordingly made up, one lot containing 5 mg. per cake and one lot 10 mg. per cake. As the larger amount was only mildly toxic, it was decided to use this dose in combination with the other drugs under question.

In the control experiments feeding unmedicated cakes mice lived 26, 36, 48, and 57 days, or an average of 41.75 days. In the tables given below are recorded in detail the results with the several drugs used:

TABLE XV.—*Control: Feeding mice cakes containing sodium nitrite 10 mg. each.*

Date.	Weight of mice.			
	1.	2.	3.	4.
1909.	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Dec. 3	16.54	17.83	17.56	20.50
6	15.32	15.16	16.32	18.20
11	13.91	14.17	15.10	17.85
18	12.09	13.55	13.95	18.09
24	12.01	11.97	14.01	15.32
30	....	11.81	13.76	13.55
1910.				
Jan. 3	....	11.06	12.84	14.02
6	....	....	12.50	12.21
[illegible]	....	....	....	12.23
[illegible]	....	....	....	11.95

No. 1 dead December 26, 23 days; No. 2 dead January 6, 34 days; No. 3 dead January 9, 37 days; No. 4 dead January 17, 45 days; average, 34.75 days.

At a somewhat later date a series of mice were fed cakes containing sodium nitrite 0.010 gram and lived 15, 36, 39, and 57 days, or an average of 26.75 days.

TABLE XVI.—*Control: Feeding mice cakes containing 0.05 gram acetanilide each.*

Date.	Weight of mice.			
	1.	2.	3.	4.
1909.	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Dec. 3	18.06	16.85	18.71	16.65
4	18.06	15.57	17.17	15.68
6	16.87	13.88	14.84	14.32
8	16.46	12.25	13.45	12.51
11			11.26	10.62
16				8.80

No. 1 dead December 7, 4 days; No. 2 dead December 11, 8 days; No. 3 dead December 13, 10 days; No. 4 dead December 16, 13 days; average, 8.75 days.

In another series mice were fed upon cakes containing the same dose of acetanilide; each lived 3, 4, 6, and 8 days, or an average of 5.25 days.

TABLE XVII.—*Control: Feeding mice cakes containing 0.06 gram acetphenetidin each.*

Date.	Weight of mice.			
	1.	2.	3.	4.
1909.	*Grams.*	*Grams.*	*Grams.*	*Grams.*
December 22	13.68	12.24	15.01	12.92
24	11.51	10.60	12.61	11.51
27		9.55	11.28	10.23
30				10.98
1910.				
January 3				10.22
7				10.81
10				10.75

No. 1 dead December 27, 5 days; No. 2 dead December 29, 7 days; No. 3 dead December 29, 7 days; No. 4 dead January 11, 20 days; average, 9.75 days.

TABLE XVIII—*Control: Feeding mice cakes containing para-amidophenol 0.04 gram each.*

Date.	Weight of mice.			
	1.	2.	3.	4.
1909.	*Grams.*	*Grams.*	*Grams.*	*Grams.*
December 15	12.78	11.51	11.66	11.15
17	11.72	10.98	10.66	10.56
20	10.38	10.52	10.24	10.20
24	10.25	10.31	10.45	
27		9.84	9.88	9.80
30		9.17	9.92	9.78
1910.				
January 3		8.16	9.04	9.25
7			8.57	
10				8.84
18				8.41

No. 1 dead December 27, 12 days; No. 2 dead January 5, 21 days; No. 3 dead January 10, 26 days; No. 4 dead January 19, 35 days; average, 23.5 days.

In another series using the same amount of para-amidophenol in each cake the mice lived 13, 30, 35, and 41 days, or an average of 29.75 days.

In like manner mice were fed on cakes containing acetanilide and sodium nitrite, acetphenetidin and sodium nitrite, and para-amidophenol and sodium nitrite, using the same amounts in each cake as in the above experiments. The results of the feeding experiments are recorded in the following tables.

TABLE XIX.—*Acetanilide–sodium nitrite: Feeding mice cakes containing acetanilide 0.05 and sodium nitrite 0.01 gram each.*

Date.	Weight of mice.			
	1.	2.	3.	4.
1909.	*Grams.*	*Grams.*	*Grams.*	*Grams.*
December 3	13.00	13.69	14.50	15.58
4	12.06	12.60	13.80	13.94
6	10.48	11.30	12.58	12.00
8	9.84	10.84	11.82	11.50
11	....	....	10.84	10.42

No. 1 dead December 9, 6 days; No. 2 dead December 10, 7 days; No. 3 dead December 11, 8 days; No. 4 dead December 15, 12 days; average, 8.25 days.

In another series in which the same amounts of the two drugs were fed in cakes the mice lived 6, 6, 7, and 7 days, or an average of 6.5 days.

TABLE XX.—*Acetphenetidin–sodium nitrite: Feeding mice cakes containing acetphenetidin 0.06 and sodium nitrite 0.01 grams each.*

Date.	Weight of mice.			
	1.	2.	3.	4.
1909.	*Grams.*	*Grams.*	*Grams.*	*Grams.*
December 22	15.09	16.30	15.65	16.12
24	13.72	14.91	14.16	14.92
27	11.77	13.80	11.93	12.92
30	....	13.00	11.52	13.37
1910.				
January 3	....	....	....	14.20
7	....	....	....	12.69

No. 1 dead December 30, 8 days; No. 2 dead January 1, 10 days; No. 3 dead January 3, 12 days; No. 4 dead January 10, 19 days; average, 12.25 days.

Table XXI.—*Para-amidophenol-sodium nitrite: Feeding mice cakes containing para-amidophenol 0.04 and sodium nitrite 0.01 grams each.*

Date.	Weight of mice.			
	1.	2.	3.	4.
1909.	*Grams.*	*Grams.*	*Grams.*	*Grams.*
December 15	10.45	11.58	10.95	12.36
17			10.60	11.30
20				10.58

No. 1 dead December 17, 2 days; No. 2 dead December 17, 2 days; No. 3 dead December 19, 4 days; No. 4 dead December 21, 6 days; average, 3.5 days.

Table XXII.—*Series II, para-amidophenol, 0.04; sodium nitrite, 0.01 gram per cake.*

Date.	Weight of mice.			
	1.	2.	3.	4.
1909.	*Grams.*	*Grams.*	*Grams.*	*Grams.*
December 22	12.95	12.87	13.50	14.27
24	11.40	11.36	11.57	12.89
27				11.66

No. 1 dead December 25, 3 days; No. 2 dead December 25, 3 days; No. 3 dead December 26, 4 days; No. 4 dead December 27, 5 days; average, 3.75 days.

To make a comparison between the control and the experiments using the drugs in combination, the following summaries are given:

Summary.

ACETANILIDE-SODIUM NITRITE.

Series.	Control.			Combination: acetanilide, 0.050; sodium nitrite, 0.010.
	Plain cakes.	Sodium nitrite 0.010.	Acetanilide, 0.050.	
	Days.	*Days.*	*Days.*	*Days.*
I	26	23	4	6
	36	34	8	7
	48	37	10	8
	57	45	13	12
Average	41.75	34.75	8.75	8.25
II		15	3	6
		36	4	6
		39	6	7
		57	8	7
Average		36.75	5.25	6.5
General average	41.75	35.75	7.00	7.37

Summary—Continued.

ACETPHENETIDIN—SODIUM NITRITE.

Series.	Control.			Combination: Acetphenetidin, 0.060; sodium nitrite, 0.010.
	Plain cakes.	Sodium nitrite, 0.010.	Acetphenetidin, 0.060.	
	Days.	*Days.*	*Days.*	*Days.*
I.	26	23	5	8
	36	34	7	10
	48	37	7	12
	57	45	20	19
Average	41.75	34.75	9.75	12.25

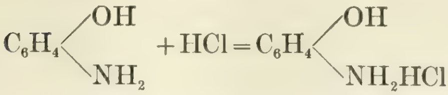
PARA-AMIDOPHENOL—SODIUM NITRITE.

Series.	Control.			Combination: Para-amidophenol, 0.040; sodium nitrite, 0.010.
	Plain cakes.	Sodium nitrite, 0.010.	Para-amidophenol, 0.040.	
	Days.	*Days.*	*Days.*	*Days.*
I.	26	23	12	2
	36	34	21	2
	48	37	26	4
	57	45	35	6
Average	41.75	34.75	23.5	3.5
II	....	15	13	3
	....	36	20	3
	....	39	35	4
	....	57	41	5
Average	....	36.75	29.75	3.75
General average	41.75	35.75	26.62	3.62

It is obvious from a comparison of these summaries that sodium nitrite, when given with acetanilide, does not markedly change its toxicity. In the case of acetphenetidin much the same result was obtained, although in this instance, basing conclusions on the one series, the toxicity was somewhat lessened by sodium nitrite. With para-amidophenol, however, the increase in toxicity is very striking when sodium nitrite is added, the combination of drugs causing death in about one-seventh the average time for para-amidophenol alone.

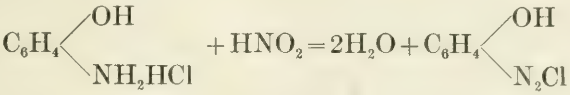
The explanation of this marked increase in toxic action rests probably upon the chemical constitution of para-amidophenol, which is a primary amine of the aromatic series. As is well known, these bodies, in the presence of nitrous acid, form diazonium compounds, the production of which is usually carried out by adding sodium nitrite to the amine in the presence of a proportionate amount of free mineral acid. Thus para-amidophenol and sodium nitrite, in the presence of hydrochloric acid, react to form nitrous acid and the amine hydrochloride:

$$\mathrm{NaNO_2 + HCl = NaCl + HNO_2}$$ and



$$\mathrm{C_6H_4}\left<\begin{matrix}\mathrm{OH}\\ \mathrm{NH_2}\end{matrix}\right. + \mathrm{HCl} = \mathrm{C_6H_4}\left<\begin{matrix}\mathrm{OH}\\ \mathrm{NH_2HCl}\end{matrix}\right.$$

The nitrous acid then reacts with the amine salt as follows to form the diazonium compound:



$$\mathrm{C_6H_4}\left<\begin{matrix}\mathrm{OH}\\ \mathrm{NH_2HCl}\end{matrix}\right. + \mathrm{HNO_2} = \mathrm{2H_2O} + \mathrm{C_6H_4}\left<\begin{matrix}\mathrm{OH}\\ \mathrm{N_2Cl}\end{matrix}\right.$$

In the cakes containing sodium nitrite and para-amidophenol a mineral acid is lacking to complete the reaction as outlined above. The gastric juice of mice is acid in reaction, however, so that it seems fair to conclude that a diazonium compound is formed in the stomach of the mice. Whether this substance exerts its toxic action as such or as a decomposition product (diazonium compounds are very unstable) it is impossible to say. The important fact in this connection is that para-amidophenol is made markedly more toxic in the presence of sodium nitrite. This reaction, however, apparently does not take place after absorption since both acetanilide and acetphenetidin, which are both decomposed in the body with the formation of para-amidophenol are rather less than more toxic when given with sodium nitrite.

That a diazonium compound was formed was further borne out by noting in some of the experiments that follow that solutions of the salt para-amidophenol hydrochloride, when added to dilute solutions of sodium nitrite, became a deep brown color. As most diazonium compounds are colored, this suggested further that not only would other primary aromatic amines react with sodium nitrite, giving colored solutions, but that other more common[a] medicinal substances, without regard to this structural formula, might also react in a similar way, at least as regards giving a change in color to the solution.

As this study was primarily concerned with possible effects of the nitrites of flour upon other drugs under such conditions as they would normally be brought together, as in the stomach, they were always dissolved in 0.1 per cent hydrochloric acid, thus giving an acidity comparable to, though weaker, than that of the normal gastric juice. Preliminary qualitative tests were first made in this way and such substances as reacted with the dilute nitrous acid thus formed were noted. Quantitative tests were then made with those drugs which gave fairly marked color changes in order to determine the limit of dilution of sodium nitrite which would visibly react in this way. The readings were made by using 50 c. c. Nessler tubes, and in all cases control experiments were carried out, omitting the addition of sodium nitrite.

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[a] With many organic drugs, i. e., pure principles, the consideration of structural formula is manifestly impossible since it is only in a comparatively few instances that such formulæ are definitely known.

The following table gives a list of such drugs as reacted in this way either with color change or with color changes and the evolution of a gas. In the estimation of the greatest dilution of nitrite which would show color the tubes were kept at a temperature of 30° for a half hour before reading, as invariably the colors became more intense on standing for some time.

Drug.	Color.	Gas.[a]	Sodium nitrite, dilution.
Acid gallic	Yellow		
Adrenaline	Red	(?)	1:10,000,000.
Anæsthesine	Yellow		
Antipyrine	Green	No	1:2,000,000.
Apomorphine	Red to green	Yes	1:20,000,000.
Atoxyl	Amber	No	1:250,000.
Benzosulphinidine	Yellow, faint		
Epinephrine	Red		
Heroin	Yellow	No	
Morphine	do	Slight	1:1,000,000.
Novocain	do	Slowly	1:500,000.
Para-amidophenol	Red to brown	No (?)	1:100,000.
Resorcinol	do	No	1:15,000,000.
Thymol	Yellow	No	1:1,000,000.
Thiosinamine	do	Yes	1:100,000.
Vanillin	do	No	1:750,000.

[a] These results are from records taken in the course of other experiments and are probably only approximately correct. At least where no gas was observed it is possible that gas may have been evolved, but so slowly as not to be easily seen in ordinary test-tube experiments.

A number of other drugs were tested in the same way, but these either did not react at all (as far as could be determined by direct examination) or reacted with the evolution of gas only. Among these were acetphenitidin, ethyl carbonate, aconitine, butylchloral hydrate, brucine, citarin, convalamarin, codeine, cinchonine, cocaine, chloralformamide, German digitalin, digitoxin, hexamethylamine, piperazine, picrotoxin, quinine, saponin, santonin, salicin, scopolamine, strychnine, salicylic acid, and veratrine.

TOXICITY EXPERIMENTS.

Toxicity experiments were carried out with a number of the drugs which had shown color reactions with sodium nitrite in the presence of dilute hydrochloric acid. In these experiments white mice were used, and while of different lots they were obtained from the same lot for the whole series of experiments on any one drug. The mice were kept in large cages and just before using were weighed and placed in separate mouse jars where they were kept until they either recovered or died from the effects of the drug. The injections in all cases, except as will be mentioned later, were made beneath the loose skin of the back, the strength of the solution being arranged so that in no case more than 0.5 c. c. was injected.

The drug to be tested was placed in a glass stoppered cylinder graduate and put into solution by adding a 0.1 per cent solution of hydrochloric acid for the control experiments and in such a solution to which had been added a small amount of sodium nitrite in the amount to be indicated in the protocols which follow in order to determine changes in toxicity. In certain of the earlier experiments the amount of nitrite added was approximately half the fatal dose of the drug in question, but dilutions up to 1 part sodium nitrite in 20,000 were used in certain instances. In certain experiments also the nitrite and the other drug in question were injected separately, either subsequently after a varying period of time or at the same time, but in a different skin area, so as to determine whether the change in toxic action would also occur after the substances had entered the circulation. It first became necessary to determine the least fatal dose of sodium nitrite for white mice, and this was found, as is shown in Table XXIII, to be approximately 0.15 mg. per gram of body weight.

TABLE XXIII.—*Control: Determination of the least fatal dose of sodium nitrite dissolved in distilled water for white mice, subcutaneous injection.*

Weight.	Dose per gram.	Result.	Time till death.
Grams.	*Mg.*		*H. m.*
20.88	0.08	—	....
16.42	.10	—	....
12.67	.12	—	....
19.87	.15	—	....
14.30	.15	+	1 20
14.52	.20	+	48
13.15	.25	+	35
14.68	.30	+	25

PARAMIDOPHENOL HYDROCHLORIDE.

Para-amidophenol hydrochloride was the first drug to be taken up in this series of experiments. Its toxicity for white mice in the absence of nitrite was first determined, and in this way it was shown that the least fatal dose was approximately 0.6 mg. per gram body weight. See Table XXIV.

TABLE XXIV.—*Control: Determination of the least fatal dose of para-amidophenol in hydrochloric acid for white mice, subcutaneous injection.*

Weight.	Dose per gram.	Result.	Time till death.
Grams.	*Mg.*		*H. m.*
15.80	0.4		....
14.31	.5	-	....
18.21	.6	+	2 30
12.83	.6	+	2 40
17.52	.7	-	....
14.63	.7	+	3 00
19.81	.8	+	2 00
17.72	1.0	+	1 55

The addition of sodium nitrite increased the toxic action of para-amidophenol very markedly when these substances were brought together in dilute hydrochloric acid a short time previous to their injection. Approximately one-thirtieth the least fatal dose of each proved to be lethal, as is shown in the results given in Table XXV.

TABLE XXV.—*Determination of the toxicity of para-amidophenol hydrochloride and sodium nitrite in hydrochloric acid for white mice; subcutaneous injection.*

Weight.	Dose per gram.		Result.	Time till death.
	Para-amido-phenol.[a]	Sodium nitrite.[b]		
Grams.	*Mg.*	*Mg.*		*H. m.*
18.10	0.010	0.005	–	....
20.32	.020	.005	+	2 40
20.60	.025	.0125	+	2 5
15.94	.05	.0125	+	19
17.53	.10	.025	+	12
20.36	.2	.025	+	13
20.92	.4	.05	+	6
22.60	.4	.05	+	5

[a] Control least fatal dose 0.6 mg. per gram.
[b] Control least fatal dose 0.15 mg. per gram.

In another series three mice were injected with varying doses of para-amidophenol hydrochloride and later with sodium nitrite, but in this way no greatly increased toxicity was observed, the effect being apparently only a summation of the toxic properties of the separate drugs. Nor was the result entirely unexpected, as in the alkaline blood where the drugs would be brought together the diazonium reaction would probably not take place. These experiments are summarized in the following protocols:

January 11, 1910: Mouse weight, 15.80 grams; dose para-amidophenol hydrochloride per gram=0.5 mg.; at 3 p. m., January 12, 1910, 0.06 mg. sodium nitrite per gram subcutaneously; very sick, convulsions. Recovered.

January 11, 1910: Mouse weight, 15.82 grams; dose para-amidophenol hydrochloride per gram=0.4 mg.; 20 minutes later 0.05 mg.

sodium nitrite per gram subcutaneously; very much stimulated. Dead in 3 hours.

January 11, 1910: Mouse weight, 17.37 grams; dose para-amidophenol hydrochloride per gram=0.4; one hour later 0.055 mg. sodium nitrite per gram subcutaneously. Dead about 6 hours later.

In another series two mice were injected with para-amidophenol hydrochloride under the skin of the back and at the same time with sodium nitrite beneath the skin of the abdomen. As is shown by the following protocols, in neither case was the result fatal: January 11, 1910: Mouse weight, 16.48 grams; dose para-amidophenol hydrochloride per gram=0.03 plus 0.05 sodium nitrite; no symptoms recorded; animal recovered. January 11, 1910: Mouse weight, 17.24 grams; para-amidophenol hydrochloride, 0.4 mg. per gram, sodium nitrite 0.05 mg. No symptoms recorded; animal recovered.

Antipyrine.—Antipyrine has long been considered incompatible with the nitrites. Test-tube experiments showed that a bluish-green color resulted from the mixture of these substances, and to determine the toxicity of the product thus formed the following experiments were carried out. Control experiments with antipyrine were not made, but the results obtained and reported in Bulletin 53 of this laboratory series were used. It was shown in that study that the least fatal dose of antipyrine for white mice (see p. 49, Bull. 53) was approximately 1 mg. per gram weight. For the minimum lethal dose of sodium nitrite for white mice (0.15 mg. per gram weight) see page 31. The results of the experiments with an antipyrine, sodium nitrite solution are given in the following table:

TABLE XXVI.—*Determination of the toxicity of antipyrine and sodium nitrite in hydrochloric acid for white mice; subcutaneous injection.*

Weight.	Dose per gram—		Result.	Time till death.
	Antipyrine.	Sodium nitrite.		
Grams.	*Mg.*	*Mg.*		*Minutes.*
18.23	0.25	0.0375	-	....
19.91	.33	.05		....
21.77	.42	.063	-	....
24.96	.50	.075	+	22
15.87	.60	.075	+	25

Thus it will be noted that the above combination apparently combines the toxic action of its components and is merely a summation effect, since amounts just below half the fatal dose of each is not, while amounts just half are, fatal.

Resorcinol.—A few experiments were carried out with resorcinol. To determine the least fatal dose of resorcinol uncombined 0.1, 0.2, and 0.3 mg. doses per gram weight were injected subcutaneously into white mice. The mouse receiving the largest dose died after 17 hours, 55 minutes.

Resorcinol in dilute hydrochloric acid solution plus sodium nitrite gave a product having a brown color which deepened on standing. This resorcinol, sodium nitrite product was injected into three mice. The two receiving doses representing half the fatal dose of each drug, recovered. The one receiving two-thirds the fatal dose of resorcinol plus one-half the fatal dose of sodium nitrite died after 9 hours 35 minutes, thus indicating a mere summation effect.

Caffeine.—Only a very slight color develops on the addition of sodium nitrite to caffeine citrate, but a few experiments were carried out with this combination. As controls, the least fatal dose of caffeine citrate for white mice, as determined in the experiments recorded in Bulletin 53, page 29, and the least fatal dose of sodium nitrite as determined, page 31, were used. In two experiments sodium nitrite was used in half the fatal dose combined with half the fatal dose of caffeine citrate. This mouse died in 3 hours 40 minutes. In another experiment a mouse receiving just the fatal dose of caffeine citrate combined with sodium nitrite, 1 part in 10,000, lived, but with the dose slightly larger of caffeine (0.9 mg. per gram) with 1 part sodium nitrite in 10,000 the mouse died after 30 minutes.

These experiments, therefore, suggest merely summation effect from the combination of the two drugs.

Epinephrine.—Epinephrine in dilute hydrochloric acid solution is changed from a clear to a deep red color by the addition of very small amounts of sodium nitrite. In the toxicity experiments, using these drugs in combination, mice were injected with 0.01, 0.02, and 0.03 mg. per gram weight epinephrine combined with sodium nitrite 1 part in 10,000. None of the mice died and none showed marked symptoms of intoxication, although the dose of 0.008 mg. per gram has been shown by Schultze[a] to be almost invariably fatal. Hence it may be concluded that even minute quantities of nitrites decrease the toxic action of epinephrine and epinephrine-like bodies.

Morphine.—The addition of small amounts of sodium nitrite to a dilute hydrochloric solution of morphine sulphate results in a change of color to a pale yellow to amber, depending apparently on the concentration of nitrite.

In carrying out toxicity experiments with morphine sulphate and sodium nitrite in combination, it was first necessary to determine the least fatal dose of morphine as a control. This was found to be approximately 0.6 mg. per gram of body weight. The results of such experiments are given in the following table:

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[a] Schultze: Bull. 55, Adrenalin and Adrenalin-like Bodies, Hygienic Laboratory, U. S. Public Health and Mar.-Hosp. Service, Washington, D. C.

TABLE XXVII.—*Control:* [a] *Determination of the least fatal dose of morphine sulphate in hydrochloric acid solution for white mice; subcutaneous injection.*

Weight.	Dose per gram.	Result.	Time till death.
Grams.	*Mg.*		*H. m.*
20.42	0.50	−	....
19.01	.55	−	....
21.32	.60	+	0 45
17.81	.65	+	2 00
18.37	.70	+	1 12

[a] It will be noted in this connection that mice of different lots vary considerably in their reaction to morphine. Hunt and Seidell, Bulletin 47, Hyg. Lab., U. S. P. H. and M.-H. S., giving the fatal dose as 0.16 to 0.45 mgm. per gram of body weight.

Using also as a control the least fatal dose of sodium nitrite as determined on page 31, the following experiments (see Table XXVIII) with the combined drugs were carried out.

TABLE XXVIII.—*Determination of the toxicity of morphine sulphate and sodium nitrite in dilute hydrochloric acid solution for white mice; subcutaneous injection.*

Weight.	Dose per gram.		Result.	Time till death.
	Morphine.	Sodium nitrite.		
Grams.	*Mg.*	*Mg.*		*H. m.*
15.65	0.20	0.050	+	0 40
16.66	.30	.075	+	1 05
16.50	.20	[a] 1:10,000	+	1 08
19.67	.15	1:10,000	+	0 50
14.53	.10	1:20,000	+	1 35
20.69	.075	1:20,000	−	....
18.03	.050	1:20,000	−	....

[a] Amount of nitrite in solution of morphine=parts per thousand.

From the above table it will be seen that morphine sulphate, even when combined with 1 part of sodium nitrite in 20,000 has an increased toxicity of about 600 or, on the basis of Hunt and Seidell's figures (loc. cit.), of 160 to 450 per cent.

It was wondered whether such an increased toxic action could also be developed by injecting the drugs separately so that if any chemical or synergistic action took place it would necessarily be subsequent to their absorption into the circulation. To determine this, three mice were injected with morphine sulphate dissolved in 0.1 per cent hydrochloric acid solution beneath the skin of the back and at the same time with sodium nitrite beneath the skin of the abdomen. The results proved that the toxic action was still much greater than would be the mere summation of toxic effects observed in the controls. The protocols which follow illustrate this.

Mouse No. 1: 15.37 grams weight plus 0.25 mg. morphine sulphate (back) and 0.06 mg. sodium nitrite (abdomen) per gram weight. Dead in 30 minutes.

Mouse No. 2: 20.65 grams weight plus 0.1 mg. morphine sulphate (back) and 0.03 mg. sodium nitrite (abdomen) per gram weight. Dead in 1 hour 12 minutes.

Mouse No. 3: 15.78 grams weight plus 0.075 mg. morphine sulphate (back) and 0.01 mg. sodium nitrite (abdomen) per gram weight. Recovered.

Novocaine.—Novocaine one of the few primary aromatic amines used in modern medicine, probably reacts with the nitrites in the presence of dilute hydrochloric acid to form a diazonium compound. As was noted in the case of para-amidophenol, a primary aromatic amine, such compounds may have a markedly toxic action and accordingly a series of experiments was undertaken to determine if an analogous increase in toxicity might not also occur in the case of novocaine.

The first step in determining this point was to make control experiments with novocaine uncombined with sodium nitrite, in order to determine the least fatal dose for white mice by subcutaneous injection. This dose, as is shown by the following table, was shown to be approximately 0.8 mg. per gram body weight.

TABLE XXIX.—*Control: Determination of the least fatal dose of novocaine in dilute hydrochloric acid solution for white mice; subcutaneous injection.*

Weight.	Dose per gram.	Result.	Time till death.
Grams.	*Mg.*		*H. m.*
18.88	0.30	–	....
17.63	.50	–	....
19.10	.75	–	....
21.54	.75	–	....
18.25	.85	+	1 35
18.36	.85	+	1 40

In the experiments with novocaine and sodium nitrite in combination, many irregularities appeared for which no certain explanation can be given. It is known, however, that the diazonium compounds are very unstable bodies and it is therefore quite possible that in those cases where the toxic action was most manifest that the drug had been injected at the optimum point in the changing chemical reaction. Thus, in one instance, from doses less than half the least fatal dose of the separate substances death occurred after seven minutes, while somewhat later the injection of the same solution in larger doses was without toxic effect. In other cases when much less nitrite was used death resulted from small doses while when injected somewhat later the animals recovered. A summary of these experiments is given in Table XXX.

TABLE XXX.—*Determination of the toxic action of novocaine and sodium nitrite in dilute hydrochloric acid solutions for white mice; subcutaneous injection.*

Weight.	Dose per gram.		Result.	Time till death.
	Novocaine.	Sodium nitrite.		
Grams.	*Mg.*	*Mg.*		*H. m.*
16.73	0.10	0.06	–	....
14.51	.30	.06	+	0 7
15.10	.50	.05	–	....
14.56	.10	[a] 1 : 10,000	+	19 45
18.41	.15	1 : 10,000	+	21 30
14.76	.20	1 : 10,000	–	....
16.39	.40	1 : 10,000	–	....
14.08	.60	1 : 10,000	–	....
14.82	.75	1 : 10,000	+	0 27

[a] Indicates the dilution of sodium nitrite used in making up solution.

By comparing the above table with Table XXIX it will be noted that in certain instances death resulted from doses approximately one-eighth the least fatal dose of novocaine when given alone, though the time of death was delayed. But there is evidence also from the larger number of mice which recovered that the effect was often only an approximate summation effect of the toxic effect of novocaine and nitrite.

Apomorphine.—Of the whole series of drugs experimented with, apomorphine appeared to react most actively with dilute sodium nitrite solutions. In all cases a marked evolution of gas occurred but no attempt was made at its analysis or to discover the exact chemical changes going on. Toxicity experiments demonstrated, however, that this reaction between apomorphine and sodium nitrite considerably increased the toxicity of the combination above that of a mere summation effect.

Control experiments using apomorphine hydrochloride in dilute hydrochloric acid solution showed that the least fatal dose for white mice to be approximately 0.4 mg. per gram body weight. These experiments are recorded in Table XXXI.

TABLE XXXI.—*Control: Determination of the least fatal dose of apomorphine hydrochloride for white mice; subcutaneous injection.*

Weight.	Dose per gram.	Result.	Time till death.
Grams.	*Mg.*		*H. m.*
18.48	0.2	–	....
16.76	.2	–	....
15.54	.3	–	....
14.45	.4	+	4 10
14.38	.5	+	1 20

Having thus determined the least fatal dose of apomorphine hydrochloride, a series of experiments were made with apomorphine hydrochloride and sodium nitrite in dilute hydrochloric acid solutions. The results are given in Table XXXII.

TABLE XXXII.—*Determination of the toxic action of apomorphine hydrochloride and sodium nitrite in dilute hydrochloric acid for white mice: subcutaneous injection.*

Weight.	Dose in milligrams per gram.		Result.	Time till death.
	Apomorphine HCl.	Sodium nitrite.		
Grams.				*H. m.*
19.86	0.075	[a] 1 : 5,000	−	....
17.83	.100	1 : 5.000	+	1 20
21.84	.125	1 : 5,000	+	1 40
16.28	.150	1 : 5,000	−	....
14.37	.200	1 : 10,000	+	(b)
16.31	.200	1 : 10,000	+	1 00
18.23	.250	1 : 10,000	+	1 00

[a] Indicates dilution of sodium nitrite used in making solution.
[b] Not recorded.

From this table it will be seen that the compound resulting from the action of the nitrite on the apomorphine is approximately 400 per cent more toxic than the original constituents.

To determine whether this change in character of the apomorphine would effect its emetic action, four dogs of about equal weight were used, being injected with doses ranging from 1 mg. down to a half milligram per kilogram. Two of the dogs received apomorphine and two apomorphine as changed by the addition of sodium nitrite, about 1 part in 20,000. Three days later the same dogs were again injected, but the two which served as controls in the first series were given the combination of drugs while the two which had received the combination were used as controls. No alteration in the effect of the drug on the vomiting center was apparent from these experiments, however vomiting appearing in both the control experiments, and the dogs receiving the combination of drugs at approximately the same time after injection and continuing for about the same time with approximately the same activity. Thus it may be concluded that although sodium nitrite in dilute hydrochloric acid materially increases the toxicity of apomorphine it does not apparently change its emetic action.

CONCLUSIONS.

The results of the experiments recorded in this paper may be summed up as follows:

1. The gluten of unbleached flour of the same grist is more easily digested by an artificial gastric juice than that of flours bleached with varying amounts of nitrogen peroxide.

2. Sodium nitrite, when added to an artificial gastric juice in a strength of 1 part to 5,000 to 200,000 (40 to only 1 part of nitrogen per 1,000,000), lessens its proteolytic action from a marked to a just determinable degree.

3. Alcoholic extracts made from slightly to markedly overbleached (overtreated) flour are toxic for white mice and rats when given subcutaneously. Alcoholic extracts of unbleached flour prepared in the same way are not toxic.

4. Alcoholic extracts of slightly to markedly overbleached (overtreated) flour are devoid of any marked toxic action on rabbits when given per stomach. Those from overtreated flour caused a somewhat more marked diminution in weight in one series, a negligible decrease in another.

5. Sodium nitrite, even in very dilute acid solution (up to 1 part to 20,000,000 or 1 part to 100,000,000 of nitrogen as nitrite) causes definite color and chemical changes in a large number of common medicinal substances.

6. Sodium nitrite in dilute acid solution (up to 1 part to 20,000 or 1 part to 100,000 of nitrogen as nitrite) markedly increases the toxic action of a number of medicinal substances. In certain cases no effect is produced and in others the toxicity is lessened.

The application of these results to the possible harmful effects of the artificial bleaching of flour suggests that a deleterious action does result because of the lessened digestibility of the gluten of such flour, and possibly also because of the presence of definitely toxic substances, although these, it must be admitted, are present, even in overtreated flour, in only minute amounts.

Independently of these factors, also, the bleaching process may produce harmful results on account of the presence in flour bleached by such processes of small amounts of the nitrites. This action is exerted in two ways—by a decrease in the rate of protein digestion and by changing other medicinal substances which may be taken at the same time into markedly toxic agents, or by decreasing their normal action, or possibly through the chemical change, altering entirely their therapeutic effects.

That the nitrites may enter the stomach from other sources does not alter the fact that they act thus deleteriously, and that their ingestion should be decreased as much as possible and, when other drugs are given, as absolutely as possible.

HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi.* By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

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*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

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TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

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HYGIENIC LABORATORY.—BULLETIN No. 69
JUNE, 1910

THE EFFECTS OF A RESTRICTED DIET AND OF VARIOUS DIETS UPON THE RESISTANCE OF ANIMALS TO CERTAIN POISONS

BY

REID HUNT

WASHINGTON
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INTRODUCTION.

Although the movement in medicine which has resulted in the substitution of attention to the diet for the use of drugs in many conditions has led to highly satisfactory results in the treatment of a number of diseases (notably those of the alimentary canal and of diabetes mellitus) it can scarcely be said that even the theoretical basis for the choice of the various foods in health and disease has been laid. In other words, although there is a vast accumulation of the most accurate knowledge of foods from the dynamic and economic points of view, little is known of the specific action of the various foods.[a] Facts are, however, rapidly accumulating which indicate that the latter is one of the most promising fields for future study in dietetics. Thus there have been many experiments performed on animals which showed the inadequacy of certain artificial or one-sided diets, although these contained "proteins," "fats," "carbohydrates," and "salts" in sufficient amount and a surplus of calories. Efforts have also been made to explain the failure of certain of these diets to maintain growth or body weight by the absence from them of certain materials (organic or inorganic) necessary for the formation of nucleins, lecithins, certain hormones, etc. The failure of certain proteins or mixtures of amino-acids to maintain growth or nitrogenous equilibrium has been found to be due to the absence from such proteins or mixtures of certain amino-acids. The relation of the animo-acids of different proteins to the formation of sugar and the diminution of acetonuria after carbohydrate withdrawal is also being investigated. There are also physiological observations of a positive character indicating that certain constituents of foods have an effect upon growth greater than that which would be indicated by their caloric value; this is especially true of lecithin.[b] There has also been much discussion as to the effect of various diets upon the structure of the thyroid[c] and to a less extent of other glands.[d]

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[a] More is known as to the specific effects of food in the growth and fertility of lower animals, the production of milk, the value of the meat produced, etc.

[b] Cf., e. g., Haitai. Amer. Jour. of Physiol., 1903, 10, p. 56.

[c] Watson. Lancet, 1905, I, p. 347; 1907, I, p. 985; 1907, II, p. 251; 1909, II, pp. 1382 and 1707; Quarterly Jour. of Physiol., 1909, 2, p. 383. Forsythe. Lancet, 1907, II, p. 152; 1909, II, pp. 1632 and 1774; Jour. of Physiol., 1907, 35, p. 11. Fordyce. Brit. Med. Jour., 1907, I, p. 619.

[d] Schepelmann. Archiv. für Entwicklungsmechanik, 1907, 23, p. 183.

There are, further, a number of clinical observations indicating that certain foods have important specific effects. Thus certain cases of diabetes mellitus are much benefited by oatmeal; the attempts to explain this action on the supposition that the starch of oats is different from that of other cereals are unsatisfactory. There can be little doubt that some specific action (i. e., an action independent of its fuel value) should be attributed to the oatmeal.

Several writers have called attention to differences in the susceptibility of different races of mice and rats to transplantable tumors. In a few cases these differences seem to have been traced to the influences of heredity.[a] In others they were attributed to differences in diet, [b] but whereas in Haaland's experiments the most susceptible mice (to a certain sarcoma) were those which had received a diet of hempseed, bread, milk, and some oats, and the least susceptible those which had received bread and oats, in Stahr's experiments mice which had received a diet of hempseed and milk were less susceptible than those which had had a diet of bread and water. Careful studies on this subject with especial reference to changes in weight and rate of growth (factors which seem, according to Mareschi[c] to be very important and which also seem important in my experiments upon the influence of diet upon the resistance to certain poisons) may lead to important results. Jensen [d] has suggested that it may be possible to influence favorably, by diet, metastases and recurrences.

Certain diseases (beriberi and pellagra) seem to be closely associated with and perhaps dependent upon the use of certain articles of diet (rice and maize); the latter may be "spoiled," but nevertheless the effects may properly be called specific, since other "spoiled" food does not have a similar effect.

Wherry[e] found guinea pigs made scorbutic by an exclusive diet of barley and water to show a greater degree of hemorrhagic extravasation when inoculated with plague than is usually seen.

In the course of numerous experiments performed, for the most part, for other purposes, I have observed some very striking alterations in the resistance of animals to certain poisons produced entirely by changes in diet. Thus of animals kept upon diets such as are in daily use by man some were able to resist many (up to 40) times the amount of certain poisons fatal to animals kept upon other diets. Although the results with certain of these poisons probably have no relation to man, yet they show that man is doubtless daily producing profound effects upon his resisting power to poisons and diseases, a knowledge of some of which may be of much practical importance. And it seems certain that by the use of some of these reactions changes in metabolism, which are not recognized by the methods ordinarily employed, may be detected.

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[a] Tyzzer. Jour. of Med. Res., 1909, 21, p. 519.

[b] Haaland. Berl. kl. Wochenschr., 1907, 44, p. 713; Stahr, Centrbl. f. Allgem. Path. und pathol. Anat., 1909, 20, p. 628.

[c] Mareschi. Zeitsch. f. Immunitätsforsch. u. exp. Ther., 1909, 2, (1), p. 651. Cf. also Bashford and Russell, Lancet, 1910, 1, p. 782.

[d] Jensen. Zeitsch. f. Krebsforsch., 1909, 7, p. 284.

[e] Wherry. Jour. Infectious Diseases, 1909, 6, p. 564.

Although the experiments are very incomplete, they may have value in suggesting new lines of research and new points of view for analyzing some of the problems now vaguely referred to as "the resisting power of the individual," "constitution," "diathesis," etc.

THE EFFECTS OF A RESTRICTED DIET AND OF VARIOUS DIETS UPON THE RESISTANCE OF ANIMALS TO CERTAIN POISONS.[a]

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Part I.

THE EFFECT OF A RESTRICTED DIET UPON THE RESISTANCE OF ANIMALS TO CERTAIN POISONS.

A. HISTORICAL.

The great practical value of an intimate knowledge of the metabolic processes which occur in partial inanition is obvious; as von Noorden[b] has remarked, no one not familiar with these processes can form a correct judgment as to which of the alterations in metabolism in a given disease are to be attributed to the latter and which to simple inanition.

As is well known, however, some fundamental problems in this connection are still in dispute. Thus the question whether the body in inanition can learn to use less material and work more economically is not definitely settled. It seemed probable that a study of the resistance to poisons of animals in a condition of partial inanition would throw some light upon some of these problems.

The literature bearing directly upon this subject is very small; Mansfeld[c] has recently summarized the greater part of it as follows: Delafoy found that starving frogs are much more sensitive to strychnine than are normal frogs. Lewin stated that starving animals are more resistant to quinine, atropine, and nicotine than are well nourished ones. Roger found the same when quinine and atropine were injected into a peripheral vein but found the reverse to hold when the drugs were injected into the portal vein. Jordan found digitalin to be more toxic to starving dogs. Adducco reported that cocaine, strychnine, and phenol are much more poisonous to starving than to well nourished dogs.

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[a] Manuscript submitted for publication June 3, 1910.

[b] von Noorden. Handbuch der Pathologie des Stoffwechsels, 2d ed., 1906, vol. 1, p. 480.

[c] Mansfeld. Archives internat. de Pharmacodynamie et de Thérapie, 1905, 15, p. 467; Mansfeld and Fejes, ibid., 1907, 17, p. 347.

Mansfeld himself found that starving rabbits are much more susceptible to chloral hydrate, paraldehyde, and morphine than were well nourished ones. Starvation did not cause an increased susceptibility to ethyl alcohol, amylene hydrate, and ethyl urethane. Mansfeld explained this difference in terms of the Meyer-Overton hypothesis.

Lo Monaco and Trambusti [a] reported that well nourished animals are less resistant to phosphorus than are poorly nourished ones.

Salant and Rieger [b] found the resistance of rabbits to caffeine to be diminished when the animals were starved for four or five days; the fatal dose was about 30 per cent less than in well fed rabbits.

B. EXPERIMENTAL.

I. Experiments with Acetonitrile.

The poison with which the most striking results were obtained was acetonitrile (CH_3CN). A consideration of the pharmacological action of this poison led to the belief that a study of the influences affecting its toxicity would be more suggestive than that with many other and better known poisons. The chief of these considerations was this: Most of the ordinary poisons undergo no marked chemical changes before exerting their toxic effects although the changes which they may subsequently undergo are often of much interest and their study has thrown important light on problems of metabolism. Acetonitrile, however, according to the generally accepted view of Heymans and Masoin, is poisonous only, or largely, as a result of the formation from it of hydrocyanic acid.[c] I have given arguments in earlier papers for the view that the formation of hydrocyanic acid is due to certain processes of metabolism which may be modified by drugs and diet. Thus, from a study of the toxicity of a number of nitriles of both the aliphatic and aromatic series, I was led to the view that, in the formation of hydrocyanic acid from acetonitrile, processes of oxidation (by which the methyl group is oxidized to formic acid) are probably involved. Believing that the tolerance for alcohol is probably due to the body's acquiring an increased power to oxidize the ethyl group of this substance,[a] I was led to the hypothesis that animals which had received alcohol for some time would be able to oxidize the methyl group of acetonitrile more rapidly, thus freeing a larger amount of hydrocyanic acid, and that such animals would therefore be more susceptible to acetonitrile than would normal ones. Experiments showed that animals which had received alcohol for some time were more susceptible to acetronitrile, and also that they decomposed a larger proportion of this substance than did the controls.

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[a] Centralbl. f. innere Medizin, 1894, 15, p. 701.

[b] Proceedings of Amer. Soc. for Pharmacol. and Exp. Therapeutics, Jour. of Pharmacol. and Exp. Therapeutics, 1910, vol. 1, p. 572.

[c] The literature on this subject is given in Archives internationales de Pharmacodynamie et de Thérapie, 1904, 12, p. 447, and Bulletins 33 and 47, of the Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

Similar reasoning led to experiments with this nitrile in partial inanition. It was thought probable that in this condition the intensity of certain processes of oxidation may be lowered and that consequently animals on a restricted diet would decompose less acetonitrile than do normal animals and consequently be more resistant to the poison. It was thought that a positive result would be of special interest for it would probably be an example of specific lowering of metabolism,[b] for there is little evidence that general metabolism is lowered in inanition.[c] As will be shown, the results support the hypothesis.

(*a*) EXPERIMENTS ON MICE.

Most of the mice used were bred in the laboratory; they were carefully weighed and the acetonitrile (dissolved in water) injected subcutaneously in proportion to body weight.

In the first group of experiments the food consisted of oats; some of the mice were given all they would eat, whereas others were given but a very small amount daily. Only a few experiments, viz, those in which the dose of acetonitrile was near the fatal dose, are cited.

Series 1.

A. CONTROLS. (v, 68.)

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
Feb. 16	20.46	Oats and water.
Feb. 24	18.12	
Feb. 27	17.45	Acetonitrile, 8.73 mgms., i. e., 0.5 mgm. per gm. mouse; survived.
Feb. 16	24.82	Oats and water begun.
Feb. 24	21.52	
Feb. 27	21.57	Acetonitrile, 14.02 mgms., i. e., 0.65 mgm. per gm. mouse; died, 4½ hours.

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[a] The correctness of this hypothesis has recently been proved by Pringsheim (Biochem. Zeitschr., 1906, 12, p. 143), who found that animals accustomed to alcohol oxidized a given amount in two-thirds the time required by those unaccustomed to it.

[b] Brugsch and Hirsch (Zeitschf. für exp. Pathol., 1907, 4, p. 947) found the assimilation limit for d-l-alanin to be much lower in hunger than normally.

[c] von Noorden. Handbuch der Pathologie des Stoffwechsels, 2d ed., 1906, vol. 1, p. 480; cf. Staehelin, Deutsche med. Wochenschr., 1909, 35, p. 609.

B. RESTRICTED FOOD. (v. 44-45.)

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
Feb. 16	23.16	Limited diet commenced.
Feb. 21	18.85	
Feb. 27	18.25	Acetonitrile, 20.08 mgms., i. e., 1.1 mgms. per gm. mouse; survived.
Feb. 16	19.11	Diet as above.
Feb. 21	16.02	
Feb. 27	14.02	Acetonitrile, 22.4 mgms., i. e., 1.6 mgms. per gm. mouse; survived.
Feb. 16	18.15	Diet as above.
Feb. 21	15.35	
Feb. 27	13.25	
Feb. 28	13.57	Acetonitrile 27.14 mgms., i. e., 2 mgms. per gm. mouse; survived.

The first and third of the mice of "B" were placed on a diet of oats soaked in ethyl alcohol the strength of which was gradually increased to 45 per cent; their weights increased in the course of 5 weeks to 19.02 and 19.31 grams, respectively, but they died from 0.3 and 0.35 mgm. acetonitrile per gm. mouse. Other mice which had received the full diet of oats and water for the same period recovered from 0.55 and 0.6 mgm. per gm. mouse.

Series 2.

A. CONTROL. (v, 82.)

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
June 7	24.85	Oats and water.
June 14	21.61	
June 19	21.72	Acetonitrile, 15.23 mgms., i. e., 0.7 mgm. per gm. mouse; died 2¾ hours.

B. RESTRICTED DIET.

1905.	*Grams.*	
June 7	29.61	Limited diet begun.
June 12	25.31	
June 16	21.21	
June 19	17.35	Acetonitrile, 20.82 mgm., i. e., 1.2 mgms. per gm. mouse; survived.
June 20	— —	Diet changed to ham and cheese.
June 30	20.01	
July 3	21.32	
July 11	25.12	Acetonitrile, 15.09 mgms., i. e., 0.6 mgm. per gm. mouse; died, 1½ to 2 hours.

Series 3.

A. CONTROL. (v, 84.)

1905.	*Grams.*	
June 7	20.85	Oats and water.
June 14	18.31	
June 25	18.26	Acetonitrile, 12.78 mgms., i. e., 0.7 mgm. per gm. mouse; died, 11 hours.

B. LIMITED DIET.

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
June 9	16.61	Limited diet commenced.
June 16	14.91	
June 24	14.12	
June 27	13.35	Acetonitrile, 13.55 mgms., i. e., 1 mgm. per gm. mouse; survived.
June 7	18.21	Diet as above.
June 16	16.51	
June 24	14.82	
June 26	14.45	Acetonitrile, 17.34 mgms., i. e., 1.2 mgm. per gm. mouse; survived.
June 7	24.55	Diet as above.
June 16	20.02	
June 25	16.60	Acetonitrile, 26.56 mgms., i. e., 1.6 mgm. per gm. mouse; died, 17 hours.

Series 4.

A. CONTROLS. (v, 85.)

1905.	*Grams.*	
Sept. 19	17.85	Oats and water.
Sept. 25	18.31	
Oct. 1	17.13	Acetonitrile, 8.57 mgms., i. e., 0.5 mgm. per gm. mouse; survived.
Sept. 19	15.80	Diet as above.
Sept. 25	14.92	
Oct. 2	14.61	Acetonitrile, 8.04 mgms., i. e., 0.55 mgm. per gm. mouse; died, 3 hours.

B. RESTRICTED DIET. (v, 85.)

1905.	*Grams.*	
Sept. 19	16.20	Limited diet begun.
Sept. 26	14.33	
Oct. 1	11.60	Acetonitrile, 9.33 mgms., i. e., 0.8 mgm. per gm. mouse; survived.
Sept. 19	17.66	Diet as above.
Sept. 26	14.03	
Oct. 2	12.85	Acetonitrile, 10.92 mgms., i. e., 0.85 mgm. per gm. mouse; survived.
Sept. 19	18.12	Diet as above.
Sept. 26	16.22	
Oct. 2	12.31	Acetonitrile, 12.31 mgms., i. e., 1 mgm. per gm. mouse; died, 1¼ hours.

In the following group of experiments the diet consisted of "cakes" made from cracker dust and water; the controls received all they would eat (between 3 and 4 gms. daily), whereas those on the limited diet received but 1 gm. per day. The resistance to acetonitrile of mice which have received a diet of cakes is much less than that of those which have had oats.

Series 5.

A. CONTROLS. (v, 87.)

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
Sept. 19	12.60	Cakes.
Sept. 30	13.55	
Oct. 2	12.71	Acetonitrile, 2.29 mgms., i. e., 0.18 mgm. per gm. mouse; survived.
Sept. 19	16.80	Cakes.
Sept. 30	15.45	
Oct. 2	14.81	Acetonitrile, 2.96 mgms., i. e., 0.2 mgm. per gm. mouse; died 1 hour.

Other mice of this series died from 0.22 and 0.26 mgm. acetonitrile per gm. mouse.

B. RESTRICTED DIET.

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
Sept. 19	18.87	Restricted diet begun.
Sept. 25	15.41	
Sept. 28	14.31	
Oct. 1	13.41	Acetonitrile, 6.03 mgms., i. e., 0.45 mgm. per gm. mouse; survived.
Sept. 19	23.55	Diet as above.
Sept. 25	18.51	
Sept. 28	18.05	
Oct. 1	16.71	Acetonitrile, 10.03 mgms., i. e., 0.6 mgm. per gm. mouse; survived.
Oct. 2	17.15	Cakes 4 gm.
Oct. 9	14.91	
Oct. 13	15.21	Acetonitrile, 9.13 mgms., i. e., 0.6 mgm. per gm. mouse; died 2½ hours.
Sept. 19	16.69	Restricted diet begun.
Sept. 25	13.65	
Sept. 28	12.41	
Oct. 2	12.58	Acetonitrile, 8.81 mgms., i. e., 0.7 mgm. per gm. mouse; died 3½ hours.

Series 6.

In this series the mice of one group were given alternately, for 4 or 5 days at a time, full and restricted diets. It has been stated that such a method of feeding leads to a greater increase in weight than does the use of a full diet continuously, the inference being that the body learned to be more "economical" with its food.[a]

A. CONTROLS. (v, 181.)

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
Oct. 14	15.62	Oats and cakes.
Oct. 26	17.01	
Nov.. 6	18.08	
Nov. 17	19.53	
Nov. 25	19.34	
Dec. 11	20.33	
Dec. 18	21.67	Acetonitrile, 11.92 mgms., i. e., 0.55 mgm. per gm. mouse; survived.
Oct. 14	18.22	Diet as above.
Oct. 26	17.62	
Nov. 6	17.92	
Nov. 15	17.58	
Dec. 11	19.93	
Dec. 17	21.16	Acetonitrile, 13.33 mgms., i. e., 0.63 mgm. per gm. mouse; survived.
Oct. 14	14.05	Diet as above.
Oct. 26	15.61	
Nov. 6	16.80	
Nov. 15	17.73	
Nov. 21	18.12	
Dec. 2	17.28	Acetonitrile, 10.87 mgms., i. e., 0.63 mgm. per gm. mouse; died 15 hours.
Oct. 14	18.43	Diet as above.
Oct. 26	22.31	
Nov. 6	23.53	
Nov. 15	23.20	
Nov. 21	23.71	
Dec. 11	24.48	
Dec. 16	24.61	Acetonitrile, 15.99 mgms., i. e., 0.65 mgm. per gm. mouse; died 2 hours.

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[a] According to Michaud (Zeitschr. f. physiol. Chem.. 1909, 59, p. 405), N-metabolism may be much lowered by alternate periods of hunger and feeding.

B. FULL AND RESTRICTED DIETS ALTERNATELY.

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
Oct. 14	17.06	Diet begun.
Oct. 17	15.85	
Oct. 18	14.45	
Oct. 23	17.15	
Oct. 25	17.71	
Oct. 27	14.95	
Nov. 3	13.85	
Nov. 6	14.81	
Nov. 10	17.45	
Nov. 15	15.70	
Nov. 21	16.66	
Nov. 24	16.40	
Nov. 28	14.35	
Dec. 2	17.10	Acetonitrile, 10.26 mgms., i. e., 0.6 mgm. per gm. mouse; survived.
Dec. 3	....	Diet continued.
Dec. 5	17.25	
Dec. 11	16.75	
Dec. 16	19.45	
Dec. 17	18.84	Acetonitrile, 16.96 mgms., i. e., 0.9 mgm. per gm. mouse; died 2 to 3 hours.
Oct. 14	16.76	Diet begun.
Oct. 17	14.75	
Oct. 18	13.35	
Oct. 23	16.85	
Oct. 25	17.85	
Oct. 27	14.82	
Nov. 3	12.75	
Nov. 6	16.40	
Nov. 10	14.51	
Nov. 15	16.02	
Nov. 21	12.63	
Nov. 28	17.05	
Dec. 2	17.35	
Dec. 4	15.72	
Dec. 11	15.75	
Dec. 16	18.45	Acetonitrile, 14.76 mgms., i. e., 0.8 mgm. per gm. mouse; survived.
Oct. 14	15.68	Diet begun.
Oct. 17	11.65	
Oct. 23	12.65	
Oct. 25	13.83	
Oct. 27	14.91	
Nov. 3	16.65	
Nov. 6	16.70	
Nov. 10	16.65	
Nov. 15	17.02	
Nov. 21	17.46	
Nov. 24	17.63	
Nov. 28	16.11	
Dec. 2	15.06	
Dec. 3	14.54	Acetonitrile, 11.63 mgms., i. e., 0.8 mgm. per gm. mouse; died in about 15 hours.

The above experiments show that the mice which had received alternately full and restricted diets were somewhat more resistant to acetonitrile than were those which had received only the unrestricted diet. The difference was not as great as when a restricted diet alone was fed. If the increase in weight of the mice in the two groups be calculated in percentage the following figures are obtained:

Unrestricted diet: 38 per cent, 16 per cent, 33 per cent, 28 per cent.

Full and restricted diets alternately: 14 per cent, 10 per cent, 12 per cent.

The mice on the unrestricted diet had increased in weight much more than those on the alternately full and restricted diet.

That the increased resistance to acetonitrile of the mice which had received the restricted diet was not due simply to a loss of weight is suggested by those experiments in which mice receiving a full diet also lost weight but did not show an increased resistance to the poison. Many experiments will be quoted in later parts of this paper in which a marked loss of weight occurred on various diets (cheese e. g.) without there being such an increased resistance. Moreover, the administration of ethyl alcohol frequently causes a loss of weight, but the resistance to acetonitrile is distinctly lowered.

Other illustrations of marked losses of weight without an increased resistance to acetonitrile are as follows:

Hydrated chloral.

Series 7.

A. CONTROLS. (IV, 171.)

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
Jan. 9	18.21	Oats and water.
Jan. 13	17.02	
Jan. 21	18.41	Acetonitrile, 6.44 mgms., i. e., 0.35 mgm. per gm. mouse; survived.
Jan. 9	14.51	Oats and water.
Jan. 13	14.42	
Jan. 22	15.61	Acetonitrile, 6.24 mgms., i. e., 0.4 mgm. per gm. mouse; died in 17 hours.

B. HYDRATED CHLORAL.

1905.		
Jan. 9	17.92	Hydrated chloral, 5 per cent, on oats.
Jan. 13	15.45	
Jan. 16	14.13	
Jan. 22	13.71	Acetonitrile, 4.1 mgms., i. e., 0.3 mgm. per gm. mouse; survived.
Jan. 9	19.25	Hydrated chloral as above.
Jan. 13	17.01	
Jan. 16	16.61	
Jan. 23	15.55	Acetonitrile, 5.44 mgms., i. e., 0.35 mgm. per gm. mouse; died in 11 hours.

It is possible, however, that in certain cases of increased resistance to acetonitrile caused by the administration of other poisons the loss of weight caused by the latter may be a factor.

The above experiments show very clearly that mice which have received a restricted diet are more resistant to acetonitrile than are those which have received an unrestricted diet. This result might be due to either of two causes, (1) the processes by which acetonitrile is decomposed in the body with the formation of hydrocyanic acid may be less active in inanition, or (2) inanition may render the mice less susceptible to the hydrocyanic acid. The first and more probable suggestion will be discussed later; that the second suggestion is not applicable is shown by the following experiment with nitroprussiate of soda, a poison which very readily liberates hydrocyanic acid in the body:

Series 8.

A. CONTROL.

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
Oct. 3	19.75	Cakes, all the mice would eat.
Oct. 9	18.62	
Oct. 14	18.45	Nitroprussiate of soda, 0.148 mgm., i. e., 0.008 mgm. per gm. mouse; survived.

B. RESTRICTED DIET.

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
Oct. 3	20.82	Cakes, 1 gm. daily.
Oct. 5	19.01	
Oct. 7	17.22	
Oct. 9	16.21	
Oct. 12	16.25	
Oct. 14	15.55	Nitroprussiate of soda, 0.14 mgm., i. e., 0.009 mgm. per gm. mouse; died, in 1½ hours.

(b) EXPERIMENTS WITH GUINEA PIGS.

These experiments were performed primarily to determine whether there was a difference in the amounts of sulphocyanate excreted, after the injection of acetonitrile, between normally fed guinea pigs and those which had received a restricted diet. An increased excretion of sulphocyanate probably means an increased breaking up of the acetonitrile molecule and it was hoped that in this way something could be learned concerning the processes of metabolism of animals kept upon a restricted diet. The diet was in all cases qualitatively the same, but less was given to those on the restricted diet; the animals were weighed every other day and only sufficient food given to prevent a marked loss of weight. As the animals were not full grown the controls increased considerably in weight. In the first experiment the sulphocyanate was not determined quantitatively, but an approximate estimation of the relative amounts excreted was obtained by comparing the intensity of the ferric chloride reaction.

Series 9.

A. CONTROL. (v, 152.) FULL, MIXED DIET (CARROTS AND CABBAGE).

Date.	Weight of guinea pig.	Remarks.
1905.	*Grams.*	
Feb. 23	490	
Mar. 9	565	
Mar. 20	675	Acetonitrile, 168.8 mgms., i. e., 0.25 mgm. per gm. guinea pig; survived.

The urine gave an intense reaction with ferric chloride on both the first and second days after the injection of the acetonitrile.

B. RESTRICTED DIET. (v, 116.)

Date.	Weight of guinea pig.	Remarks.
1905.	*Grams.*	
Feb. 21	382	
Mar. 3	430	Restricted diet begun.
Mar. 6	405	
Mar. 9	407	
Mar. 14	376	
Mar. 17	377	
Mar. 20	325	Acetonitrile, 81.25 mgms., i. e., 0.25 mgm. per gm. guinea pig: survived.

The urine excreted in the first 24 hours, although abundant, gave no reaction whatever with ferric chloride; that for the second 24 hours gave an intense reaction.

In the following experiments the sulphocyanate was determined by Lang's [a] method.

Series 10.

In this experiment, after the injection of the acetonitrile, the feeding was reversed; i. e., the guinea pig which had received an unrestricted diet was placed on a restricted diet, and vice versa. After about two weeks sodium sulphocyanate was administered to each in order to determine whether there was any difference in the ability of the animals to excrete preformed sulphocyanate.

A. CONTROL. UNRESTRICTED DIET. (v, 117.)

Date.	Weight of guinea pig.	Remarks.
1905.	*Grams.*	
Oct. 17	355	
Oct. 28	400	
Nov. 3	425	
Nov. 15	490	
Nov. 22	495	Acetonitrile, 99 mgms., i. e., 0.2 mgm. per gm. guinea pig; survived. In the first five days after the injection of the acetonitrile the urine contained 38.7 mgms. cyanogen (corresponding to about 62 mgms. acetonitrile., i. e., about 63 per cent of the cyanogen of the nitrile had appeared in the urine).
Nov. 27	. . . .	Restricted diet begun.
Dec. 4	475	
Dec. 9	475	
Dec. 11	450	Sodium sulphocyanate, 180 mgms., i. e., 0.4 mgm. per gm. animal, injected subcutaneously; survived. In the following three days 49 mgms. of cyanogen (= 151.9 mgms. NaCNS, or 84.4 per cent of the amount injected) was excreted in the urine.

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[a] Lang, Archiv. für exper. Path. und Pharmakol, 1894, v. 34, p. 247.

B. RESTRICTED DIET. (v, 117.)

Date.	Weight of guinea pig.	Remarks.
1905.	*Grams.*	
Oct. 21	385	Restricted diet begun.
Oct. 30	400	
Nov. 3	395	
Nov. 8	390	
Nov. 15	410	
Nov. 22	410	Acetonitrile, 82 mgms., i. e., 0.2 mgm. per gm. guinea pig; survived. The urine of the five days following the injection of the nitrile contained 23.7 mgms. cyanogen; this corresponds to 38 mgms. of acetonitrile. Hence about 46 per cent of the cyanogen of the nitrile appeared in the urine as sulphocyanate.
Nov. 27	....	Unrestricted diet begun.
Dec. 4	460	
Dec. 9	500	
Dec. 11	495	Sodium sulphocyanate, 198 mgms., i. e., 0.4 mgm. per gm. animal; survived. In the following three days 49 mgms. cyanogen (=151.9 mgms. NaCNS, or 77 per cent of the amount injected) was secreted in the urine.

These experiments show (1) that the guinea pig upon a restricted diet excreted in the urine less sulphocyanate, after the administration of acetonitrile, than did the one upon an unrestricted diet, and (2) that there was not a distinct difference between the amounts of sulphocyanate excreted in the two cases after the administration of the sodium salt. Hence it may be concluded that the increased excretion in the former case was due to an increased formation of the sulphocyanate and not to any differences in the rate of excretion or destruction of the sulphocyanate which was formed.

Series 11.

A. CONTROL. UNRESTRICTED DIET. (v, 104.)

Date.	Weight of guinea pig.	Remarks.
1905.	*Grams.*	
Feb. 14	380	Acetonitrile, 76 mgms., i. e., 0.2 mgm. per gm. animal; died in 13 hours.

B. LIMITED DIET. (v, 118.)

Date.	Weight of guinea pig.	Remarks.
1905.	*Grams.*	
Jan. 30	415	Restricted diet begun.
Feb. 2	401	
Feb. 8	376	
Feb. 14	327	Acetonitrile, 65.4 mgms., i. e., 0.2 mgm. per gm. animal; survived.

The urine of "B" for the first 24 hours gave but a faint reaction for sulphocyanate; with animals on an unrestricted diet the first 24 hours urine gave, as a rule, an intense reaction.

Series 12.

The following experiments were conducted as above, except that the resistance of the animals toward another cyanogen compound, viz., nitroprussiate of soda, was first tested. The dose of the nitroprussiate of soda was but slightly less than the one usually fatal to guinea pigs; if either guinea pig had had a markedly decreased resistance to this poison it would probably have died.

A. CONTROL. (v, 98.)

Date.	Weight of guinea pig.	Remarks.
1905.	*Grams.*	
Sept. 26	335	Unrestricted diet.
Oct. 2	362	
Oct. 13	390	
Oct. 23	430	
Oct. 28	480	
Nov. 3	490	
Nov. 6	495	Nitroprussiate of soda, 4.95 mgms., i. e., 0.01 mgm. per gm. animal; survived.
Nov. 7	......	Unrestricted diet continued.
Nov. 8	495	
Nov. 15	500	
Nov. 21	520	Acetonitrile, 130 mgms., i. e., 0.25 mgm. per gm. animal; died in 13 hours.

B. RESTRICTED DIET. (v. 98.)

1905.	*Grams.*	
Sept. 26	358	Restricted diet begun.
Sept. 28	392	
Oct. 2	397	
Oct. 6	355	
Oct. 15	350	
Oct. 19	350	
Oct. 23	380	
Nov. 3	375	
Nov. 6	365	Nitroprussiate of soda, 3.65 mgms., i. e., 0.01 mgm. per gm. animal; survived.
Nov. 7	......	Restricted diet continued.
Nov. 8	365	
Nov. 11	360	
Nov. 18	390	
Nov. 21	365	Acetonitrile, 91.25 mgms., i. e., 0.25 mgm. per gm. animal; survived. The urine for the 4 days following the injection of the acetonitrile contained 18 mgms. cyanogen, i. e., 31.5 per cent of the cyanogen of the nitrile was excreted as sulphocyanate.
Nov. 27	..	Unrestricted diet begun.
Dec. 4	445	
Dec. 14	480	Acetonitrile, 91.25 mgms., i.e., 0.19 mgm. per gm. animal; died in 12 hours. The urine gave a strong reaction with ferric chloride.

Thus, with equal doses of acetonitrile the guinea pig on an unrestricted diet died while that on the restricted diet recovered. Later when the animal which had received the restricted diet was placed upon an unrestricted diet it died from a dose of acetonitrile smaller, per gm. body weight, than that from which it had recovered when it was receiving a limited diet.

The excretion of sulphocyanate after the injection of acetonitrile into the guinea pig which had received the limited diet was less (31.5 per cent of the cyanogen injected) than that which is usually found in the case of animals on an unrestricted diet.

Summary.—The above experiments show (1) that guinea pigs which have been kept upon a restricted diet are more resistant to acetonitrile than are those which have received an unrestricted diet, and (2) that in the former less of the cyanogen of the acetonitrile is execreted as sulphocyanate. The increased resistance to the poison and the diminished execretion of sulphocyanate are probably both dependent upon a lessened breaking up of the acetonitrile molecule and this again upon a diminution in the intensity of those processes of metabolism by which the acetonitrile molecule is decomposed. The body "spares" the acetonitrile molecule in hunger as it does the protein molecule and the fundamental processes may be the same in the two cases.[a]

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[a] Experiments were made with acetonitrile under a number of other conditions and with a number of poisons which have at different times been supposed to alter some processes of physiological oxidation; the results were essentially negative. Thus chloroform water was fed or injected subcutaneously into mice and guinea pigs and their resistance to acetonitrile tested; no change was noted. Negative results were also obtained with quinine, sodium bicarbonate, and carbon monoxide, and with mice kept in pure oxygen or in a greatly rarefied atmosphere. Mice kept in a cage with a wheel upon which they exercised a great deal seemed to be slightly less resistant to acetonitrile than were those kept in jars where there was little opportunity of exercise.

The effects of inanition and several drugs were tested upon the action of another poison, methyl alcohol, the toxicity of which I have been led (Johns Hopkins Hosp. Bull., 1902, 13, p. 213) to believe is due in part to the formation in the body of secondary toxic substances; the results were negative.

PART II.

THE EFFECTS OF VARIOUS DIETS UPON THE RESISTANCE OF ANIMALS TO CERTAIN POISONS.

A. HISTORICAL.

Although much has been written on the effect of different diets upon some of the changes in metabolism caused by poisons, and there are a number of observations on the effect of diet upon the changes undergone by poisons in the body,[a] but little seems to have been done upon the resistance to poisons caused by different diets. In fact the only experiments bearing directly upon this subject with which I am acquainted are those of Foster[b] upon the resistance of dogs to ricin: Of three dogs which had been kept upon a high plane of protein nutrition all died from 1 mgm. ricin per kilo, whereas of 3 kept on medium or low planes of protein nutrition 2 survived this dose.[c]

B. EXPERIMENTAL.

1. EXPERIMENTS WITH ACETONITRILE.

Although I have found diet to produce marked changes in the resistance of animals to some of the better-known poisons (such as morphine, cocaine, atropine, etc.) the most striking and, from a theoretical standpoint, the most interesting results were obtained with acetonitrile. This was the result anticipated for the reasons given in Part I, viz, that the formation of hydrocyanic acid from acetonitrile is intimately connected with processes of metabolism and these again with the character of the diet. Moreover, I have shown in previous publications[d] that the toxicity of acetonitrile is closely related to one of the most important glands of internal secretion (the thyroid) and it was of especial interest to determine by means of the reactions based upon this relation whether it is possible to influence the activity of this gland by diet.

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[a] Abderhalden and Brahm (Zeit. f. physiol. Chem., 1909, 62, p. 133), for example, find that dogs which have received only milk secrete no methyl pyridine after the administration of pyridine, but that they rapidly acquire this power after the feeding of meat and retain it even when the milk diet is resumed.

[b] Foster. Proc. Soc. Exp. Biol. and Med., 1909, 6, p. 61

[c] But cf. Foster, Jour. of Biolog. chem., May, 1910, v. 7, p. 379.

[d] Bulletin 47, Hygienic Laboratory, 1909.

Before describing the experiments a few of the conditions which are known to influence the resistance of animals to acetonitrile may be referred to briefly. The more important of these are:

Thyroid.—Numerous experiments have shown that minute amounts of thyroid fed to mice for a short time markedly increase the resistance of these animals to acetonitrile. The opposite effect, viz, an increased susceptibility to this poison is produced when thyroid is fed to rats [a] and guinea pigs.

Iodine compounds.—The administration of iodine compounds to animals has an effect (if it has any at all) upon their resistance to acetonitrile similar to but far less marked than that of thyroid, i. e., it increases the resistance of mice but diminishes that of rats and guinea pigs. I have given reasons elsewhere for believing that the effect of iodine in these cases is exerted through the thyroid gland.[b]

Sulphur.—Lang [c] found sodium thiosulphate to protect animals, to a certain extent, against hydrocyanic acid; Heymans [d] and his co-workers found it to have a similar, but often more marked, action against a number of nitriles. I found thialdine, carbothialdine, and potassium xanthogenate to have an antagonistic action toward a number of nitriles; a number of other sulphur compounds had little or no such action.[e]

In most of these experiments the sulphur compounds were injected subcutaneously or intravenously, and there is nothing to suggest that the sulphur which is contained in so many articles of food would have a similar protective action; that this may be the case, however, is suggested by certain experiments to be described later.

Season has, as was pointed out in a previous publication, a marked effect upon the resistance of mice to acetonitrile. This question will be discussed in later parts of this paper.

(*a*) EXPERIMENTS ON MICE.

In order to make the conditions as uniform as possible and to eliminate differences in susceptibility which might result from differences in race, previous diets, etc., the experiments were performed, with few exceptions, upon mice which had been bred in the laboratory and which had lived under as nearly as possible identical conditions.

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[a] It is a matter of some interest that animals which are supposed to be as closely related zoologically as are mice and rats should differ so markedly in some of their physiological reactions. Uhlenhuth and Weidanz (Arbeiten. aus dem kais. Ges.-Amte., 30, p. 434) and Trommsdorf (Ibid., 1909, 32, p. 560), as a result of a study of certain serum reactions, have recently reached the conclusion that these animals are not as closely related as is usually supposed.

[b] Jour. Amer. Med. Ass., 1907, 49, p. 1323.

[c] Lang. Archiv für exper. Path. u. Pharmakol., 1895, 36, p. 77.

[d] Heymans. Archives internat. de Pharmacodynamie et de Therapie, 3, pp. 77, 359; 7, p. 297; 8, p. 1.

[e] Hunt., ibid., 1904, 12, p. 447.

As the experiments were very numerous only the largest nonfatal and the smallest fatal doses are recorded in the tables. As a rule there is no difficulty in determining the fatal dose within 0.01 to 0.02 mgm. per gm. mouse; where, however, the fatal doses vary as widely as they did in these experiments many animals were used in determining the approximately fatal dose.

ACETONITRILE—MICE.

Series 13. (Jan., 1910.) (xii, 12.)

Groups of young, nearly grown mice (10 in each group) were kept upon the following diets for about 6 weeks (from December 3, 1909, to January 14, 1910). The milk and oatmeal, and the bread and oil were intimately mixed.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a Milk, raw[a]	Increased 8.3 per cent (from 15 to 16.25 gms)	0.08	0.10
b Olive oil, 1 part; bread, 3 parts.[b]	Decreased 21 per cent (from 15 to 11.85 gms.)	.21	.25
c Milk, boiled[c]	Decreased 2.7 per cent (from 15 to 14.66 gms.)	.25	.33
d. Cod liver oil, 1 part; bread, 3 parts[d]	Increased 6.7 per cent (from 15 to 16 gms.)	.30	.50
e. Oatmeal, 1 part; boiled milk, 2 parts[e]	Increased 38.4 per cent (from 15 to 20.22 gms.)	.45	.50
f. Oatmeal, 1 part; raw milk, 2 parts.	Increased 42.7 per cent (from 15 to 21.41 gms.)	1.30	1.40
g. Oatmeal, 1 part; water, 2 parts.[e]	Increased 16.3 per cent (from 15 to 17.44 gms.)	1.70	1.80
h. Bread (C) and water[b]	Increased 17.6 per cent (from 14.8 to 17.4 gms.)	2.70	3.00

[a] Two of the 10 had died before the acetonitrile was given.
[b] Four had died.
[c] Seven had died.
[d] Eight had died.
[e] One had died.

The results of these experiments will be discussed in more detail later, but reference may be made in this connection to a few points. The result with group *b* shows that a marked loss of weight may occur without an increased resistance to acetonitrile (cf. results on inanition, Part I). The results with groups *b*, *d*, and *h* show how markedly a high degree of resistance caused by one diet may be lowered by the addition of another food (in these cases oils). The results also show that raw and boiled milk may have different effects under different conditions, although both lowered the resistance caused by oatmeal alone. There was no relation between the increase in weight and the susceptibility to the poison.

The mice of groups *e*, *f*, *g*, and *h* which had survived were, on January 15, placed on a diet of raw milk; after 3 weeks their resistance to acetonitrile was again tested. The fatal doses of the previous period are placed in parentheses for comparison:

[Dose in mgm. per gm. mouse.]

Previous diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
e. Oatmeal, 1 part; boiled milk, 2 parts.	Increased 7.5 per cent (from 16.75 to 18 gms.)....	(0.45)	0.20(0.50)
f. Oatmeal, 1 part; raw milk, 2 parts.	Decreased 1.4 per cent (from 19 to 18.74 gms.)....	0.28(1.30)	.33(1.40)
g. Oatmeal, 1 part; water, 2 parts.	Increased 5.9 per cent (from 16.67 to 17.67 gms.) .	1.00(1.70)	(1.80)
h. Bread (C) and water.......	Increased 15.2 per cent (from 18.4 to 21.2 gms.)...	.13(2.70)	.15(3.00)

Thus the change to the raw milk diet very quickly and greatly reduced the resistance of all the groups except the one which had received, during the previous period, the diet of oatmeal and water. As will be shown later oatmeal usually leads to a long continued resistance. The reduction of resistance in the above experiment was especially striking in the case of the mice which had received bread in the previous period.

Series 14. (Feb., 1908.) (x, 104.)

Fifteen mice, not fully grown, were placed upon the following diets and their resistance to acetonitrile determined after about 8 weeks. The group "Oatmeal and milk" received at first no water; the milk (which had been boiled) was placed in a vessel separate from the oatmeal. Later a vessel containing water was also placed in the cage. The mice were not weighed until at the end of the experiment. The bread was from a different bakery than that used in the preceding series.

[Dose in mgm. per gm. mouse.]

Diet.	Weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
	Grams.		
a. Bread (S)..	19.70	0.65	0.70
b. Oatmeal..	20.12	1.60	1.70
c. Oatmeal and milk (boiled)........	20.35	2.10	2.40

Series 15. (Mar., 1908.) (x, 108.)

Grown mice were placed upon the following diets for about 12 weeks:

[Dose in mgm. per gm. mouse.]

Diet.	Weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
	Grams.		
a. Bread (S)	19.56	....	0.35
b. Oatmeal	22.27	2.10	2.30
c. Oatmeal and milk (boiled)	24.56	2.30	2.50

Series 16. (Mar., 1906.). (vi, 154.)

Mice were placed upon the following diets for about 4 weeks. They were weighed at frequent intervals:

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Barley	Decreased 4.4 per cent (from 21 to 20 gms.)	0.46	0.48
b. Rye	Decreased 1 per cent (from 17.96 to 17.76 gms.)	.53	.55
c. Wheat	No change (19.7 gms.)	.55	.57
d. Oats	No change (17.9 gms.)	.75	.80

Series 17. (May, 1908.) (x, 172.)

Groups of young mice (15 in each) were fed as below for 8 to 9 weeks:

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Egg yolk (boiled)	Increased 40 per cent (from 12.98 to 18.2 gms.)	0.12	0.15
b. Milk (boiled)	Increased 26 per cent (from 12.48 to 16.13 gms.)	.20	.21
c. Cheese	Increased 15 per cent (from 13.2 to 15.14 gms.)	.24	.25
d. Milk (raw)	Increased 13 per cent (from 12.84 to 14.55 gms.)	.28	.30
e. Ham (lean)	Increased 26 per cent (from 12.28 to 15.50 gms.)	.30	.31
f. Egg white (boiled) and bread (equal parts).	Increased 6.2 per cent (from 12.87 to 13.67 gms.)	.32	.35
g. Egg white (boiled) and oatmeal (equal parts).	Increased 13 per cent (from 13.25 to 15 gms.)	.50	.54
h. Bread (S)	Increased 16.4 per cent (from 12.67 to 14.75 gms.)	.80	1.00
i. Bread and oat ash[a]	Increased 5.8 per cent (from 12.67 to 13.4 gms.)	.80	.90
j. Oats	Increased 2 per cent (from 13.49 to 13.80 gms.)	1.50	1.80
k. Potatoes (boiled)	Decreased 7.6 per cent (from 12.67 to 11.67 gms.)	1.80	2.20
l. Liver, hog (boiled)	Increased 10 per cent (from 13.49 to 14.91 gms.)	2.80	3.50

[a] The ash from 100 gms. oats was intimately mixed with the bread and fed daily.

There was, in this series, quite a close parallelism between the degree of resistance and the rate of growth; the more rapid the growth the less was the resistance.

Series 18. (**July, 1908.**) (**xi, 2.**)

These experiments were performed in July, a month in which the resistance of mice to acetonitrile is always low.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Egg yolk (boiled)	Increased 24 per cent (from 15.3 to 19 gms.)	0.098	0.11
b. Egg, entire (boiled)	Increased 22 per cent (from 14.2 to 17.4 gms.)	.095	.10
c. Oats, extracted [a]	Decreased 16 per cent (from 15.5 to 13 gms.)	.190	.20
d. Brain [b] (sheep) and oatmeal (equal parts).	Increased 15 per cent (from 17 to 19.5 gms.)	.210	.22
e. Egg yolk and liver (equal parts).	Increased 23 per cent (from 15.1 to 18.6 gms.)	.290	.30
f. Oatmeal	Increased 5.7 per cent (from 15.7 to 16.6 gms.)	.400	.45
g. Kidney, sheep (boiled)	Increased 20 per cent (from 13.7 to 16.5 gms.)	.630	.64
h. Egg white and oatmeal (equal parts).	Increased 10 per cent (from 15.7 to 17.3 gms.)	.750	.80
i. Egg white and liver (equal parts).	Increased 2 per cent (from 15.1 to 15.4 gms.)	.870	.90
j. Liver, hog (boiled)	Increased 1 per cent (from 15.3 to 15.5 gms.)	1.50	1.80

[a] Residue after extraction with 96 per cent alcohol.
[b] Mice die in a few days on an exclusive diet of brain.

The combinations of foods gave results of some interest. Thus a mixture of egg white and egg yolk did not have an effect different from that of egg yolk alone. A mixture of egg white and liver led to a condition in which the resistance to acetonitrile was but one-half as great as when liver alone was given. The effect of adding egg white to oatmeal was the reverse; the mice became twice as resistant as when oatmeal alone was given. Brain added to oatmeal and egg yolk added to liver caused a lower degree of resistance than when oatmeal or liver was given alone.

With two or three exceptions there was, in this series, a fairly close parallelism between the degree of resistance and the increase in weight; the greater the latter the lower was the former.

Series 19. (**Aug., 1907.**) (**x, 102.**)

A number of adult mice were fed upon the following diets for about 5 weeks:

[Dose in mgm. per gm. mouse.]

Diet.	Weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
	Grams.		
a. Bread (S)	19.71	0.47	0.49
b. Oats	21.42	.56	.60

Series 20. (Dec., 1907.) (x, 100.)

A number of adult mice were kept upon the following diets for a period of about 4½ months. Many young mice were born; a careful record was not kept, but those receiving the diet of hemp seed and milk seemed to be the most prolific.

[Dose in mgm. per gm. mouse.]

Diet.	Weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
	Grams.		
a. Bread (S)	25.19	0.56	0.58
b. Bread and milk (raw)	19.63	.58	.59
c. Oats and milk (raw)	23.61	.65	.75
d. Hemp seed and milk (raw)	19.94	.65	....
e. Hemp seed	20.76	.68	.70
f. Oats	20.86	1.70	1.80

The milk very distinctly lowered the resistance caused by the oats.

Series 21. (Dec., 1909.) (xii, 2.)

Groups of mice, not fully grown, were fed as follows for about 6 weeks. The corn-meal mush was made by boiling a commercial white corn meal with water. Part of this mush was placed aside in a warm room and allowed to become sour before feeding. The bread was from a different bakery than that used in the immediately preceding series.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Corn-meal mush (fresh)	Decreased 3.8 per cent (from 13.80 to 13.28 gms.)	0.80	0.90
b. Corn-meal mush (sour)	Decreased 18.1 per cent (from 14.72 to 12.05 gms.)	.80	.90
c. Bread (C) and oats	Increased 26.2 per cent (from 13.8 to 17.42 gms.)	1.20	1.30

The resistance of other mice of these groups toward atropine sulphate and cocaine hydrochloride was tested; no difference was found between groups *a* and *c*, but those of group *b* were less resistant to both poisons.

Series 22. (Mar., 1908.) (x, 112.)

Mice, not fully grown, were fed as below for about 6 weeks.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Beans, "navy" (boiled)	Decreased 2.8 per cent (from 14 to 13.6 gms.)	0.95	1.00
b. Corn, whole grain	Decreased 11.4 per cent (from 14 to 12.4 gms.)	1.10	1.20
c. Peanuts (raw)	Decreased 7.1 per cent (from 14 to 13 gms.)	1.80	2.00
d. Beef (lean)	Increased 7 per cent (from 14 to 15 gms.)	1.80	2.00

Series 23. (Dec., 1909.) (xii, 11.)

Mice, not fully grown, were kept on the following diets for about 6 weeks.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Cotton-seed oil, 1 part; bread (C), 2 parts.	Decreased 41.5 per cent (from 15.4 to 9 gms.)	0.50	0.70
b. Olive oil, 1 part; bread (C), 2 parts.	Decreased 24.7 per cent (from 15.4 to 11.6 gms.)	.65	.80
c. Bread (C)	Increased 1.6 per cent (from 15.9 to 16.16 gms.)	2.20	2.50

The addition of oil to the diet led to a marked loss of weight and to a low degree of resistance.

Series 24. (Dec., 1909.) (xii, 30.)

The mice were fed as below for about 5 weeks.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Egg, entire	Increased 10.7 per cent (from 16.33 to 18.08 gms.)	0.85	0.95
b. Mush from good corn meal.	Decreased 9.7 per cent (from 16.33 to 14.75 gms.)	1.40	1.70
c. Mush from moldy corn meal.	Decreased 15.8 per cent (from 16.33 to 13.75 gms.)	2.50	.....

The resistance of mice to acetonitrile is usually high in the winter. The mice on the egg diet had not increased in weight as much as they usually did on this diet; their resistance was also higher.

Series 25. (Feb., 1910.) (xii, 34.)

The mice were fed as below for nearly 6 weeks. The corn oil was extracted with cold ether from yellow corn meal prepared in the laboratory.[a] The egg oil was extracted from the yolk of eggs with ether and alcohol; most of the lecithin was afterwards removed.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Corn oil, 1 part; bread (C), 2 parts.	Decreased 15.3 per cent (from 15.83 to 13.4 gms.)....	0.17	0.20
b. Egg oil, 1 part; bread (C), 2 parts.	Decreased 22.8 per cent (from 15.85 to 12.33 gms.)....	.18	.23
c. Olive oil, 1 part; bread (C), 2 parts.	Decreased 5.36 per cent (from 15.85 to 15 gms.)......	.35	....
d. Egg (whole)................	Increased 34.9 per cent (from 12.6 to 17 gms.)........	.35	.45
e. Bread (C)....................	No change (12.33 gms.)................................	4.30	4.40

The mice fed upon this particular kind of bread (C) had again a remarkably high degree of resistance. The addition to the bread of corn and egg oils lowered the resistance to about one-twentieth. The changes in weight caused by the egg diet and the bread and egg oil diet are interesting; the former caused a great increase in weight, whereas the latter caused a decrease. Both, however, caused a low degree of resistance. These results suggest that it is not the oil in the egg which causes the increase in weight (it is probably the lecithin); the oil may, however, be largely responsible for the lowered resistance in both cases.

Series 26. (Feb., 1910.) (xii, 38.)

Young mice, 12 in each group, were fed as below for about 37 days. The two kinds of bread used (C and S) were those used in previous experiments. The "Oat extract" was prepared by extracting oats, ground in the laboratory, with three times its weight of distilled water; two parts of this extract was mixed with one part of corn meal.

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[a] I am indebted to Dr. Norman Roberts for the preparation of this oil.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average.)	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Milk, raw [a]	Increased 3.16 per cent (from 12.33 to 12.72 gms.)	0.10	0.11
b. Milk, boiled [b]	Increased 4.65 per cent (from 12.33 to 12.9 gms.)	.15	.16
c. Corn meal, [b] 1 part; boiled milk, 2 parts.	Increased 27.33 per cent (from 12.33 to 15.7 gms.)	.50	.60
d. Bread (S) [c]	No change (12.24 gms.)	....	1.00
e. Corn meal, 1 part; raw milk, 2 parts.	Increased 37.14 per cent (from 12.33 to 16.91 gms.)	1.00	1.10
f. Corn meal, 1 part; oat extract, 2 parts. [d]	Decreased 3.56 per cent (from 12.33 to 11.8 gms.)	2.40	2.50
g. Corn meal [d]	Decreased 7.54 per cent (from 12.33 to 11.4 gms.)	3.00	3.40
h. Bread (C) [e]	Increased 4.46 per cent (from 12.33 to 12.88 gms.)	4.00	4.10

[a] One mouse had died.
[b] Two mice had died.
[c] Five mice had died.
[d] Two mice died.
[e] Four mice died.

Thus the difference between the effects of the two brands of bread noted in preceding experiments is evident here also. The addition of milk (raw or boiled) lowered the resistance caused by the corn meal, the boiled being more active in this respect than the raw milk.

Series 27. (May, 1910.) (xii, 46.)

Young mice (10 in each group) were fed as below for about 5 weeks. Twenty-five grams of corn meal were given daily; that fed to group *a* was mixed with 25 c. c. water, that to *b* with 5 c. c. of Armour's extract of red bone marrow [a] plus 20 c. c. water, that to *c* with one-fourth of an egg yolk and 25 c. c. water, that to *d* with one-fourth of an egg white and 25 c. c. water, that to *e* with 25 c. c. 20 per cent glycerin, that to *f* with 5 c. c. of Fairchild Bros. and Foster's lecithin solution [b] and 20 c. c. water, that to *g* with the ash of 100 c. c. milk and 25 c. c. water, and that to *h* with 25 c. c. 0.1 per cent solution of potassium iodide.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Corn meal [c]	Decreased 26.18 per cent (from 11.8 to 8.71 gms.)	1.80	2.00
b. Corn meal and bone marrow. [d]	Decreased 20 per cent (from 11.8 to 9.44 gms.)	1.10	1.20
c. Corn meal and egg yolk [e]	Decreased 11.01 per cent (from 11.8 to 10.5 gms.)	....	.70
d. Corn meal and egg white	Increased 8.48 per cent (from 11.8 to 12.8 gms.)	1.20	1.20
e. Corn meal and glycerin [f]	Decreased 15.25 per cent (from 11.8 to 10 gms.)	....	.70
f. Corn meal and lecithin [g]	Decreased 17.20 per cent (from 11.8 to 9.77 gms.)	1.30	1.40
g. Corn meal and milk ash [d]	Decreased 5.08 per cent (from 11.8 to 11.2 gms.)	1.40	[h] 1.00
h. Corn meal and potassium iodide. [g]	Decreased 24.83 per cent (from 11.8 to 8.87 gms.)	2.40	2.50

[a] See New and Non-Official Remedies, 1910, p. 144.
[b] Ibid., p. 117.
[c] Three mice had died.
[d] One mouse had died.
[e] Eight mice had died.
[f] Five mice had died.
[g] Two mice had died.
[h] Results irregular.

The mice which had lost the most weight were the most resistant to the poison.

The survivors from groups *b*, *d*, *f*, and *h* were placed upon a diet of eggs for about 13 days, when their resistance to acetonitrile was again determined; the fatal doses of the previous period are placed in brackets for comparison.

[Dose in mgm. per gm. mouse.]

Previous diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
b. Corn meal and bone marrow.	Increased 68.31 per cent (from 9.5 to 16 gms.)	0. 15 (1. 10)	0. 20 (1. 20)
d. Corn meal and egg white	Increased 33.19 per cent (from 12.2 to 16.25 gms.)	. 15 (1. 20)	. 25 (1. 20)
f. Corn meal and lecithin	Increased 65.95 per cent (from 9.4 to 15.6 gms.)	. 35 (1. 30)	. 45 (1. 40)
h. Corn meal and potassium iodide.	Increased 44.34 per cent (from 8.66 to 12.5 gms.)	(2. 40)	. 90 (2. 50)

Thus the diet of eggs had an effect similar to that of milk in other series; that is, it quickly lowered the resistance of the mice to acetonitrile.

A number of experiments were performed in which the mice were weighed separately, at short intervals; in many cases, after the resistance to acetonitrile had been determined, the diet was changed and later the resistance again determined.

Series 28.

A. INITIAL DIET: HAM AND AMERICAN CHEESE. (v, 158.)

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
Apr. 3	14. 42	Diet of ham and cheese begun.
Apr. 10	13. 91	
Apr. 19	14. 65	Acetonitrile, 2.93 mgms., i. e., 0.2 mgm. per gm. mouse; survived.
Apr. 20	.....	Diet changed to rice.
Apr. 26	15. 25	
May 3	15. 55	
May 4	15. 22	Acetonitrile, 6.09 mgms., i. e., 0.4 mgm. per gm. mouse; survived.
Apr. 3	12. 21	Ham and cheese diet begun.
Apr. 10	11. 81	
Apr. 18	13. 21	Acetonitrile, 3.96 mgms., i. e., 0.3 mgm. per gm. mouse; died, 2 hours.
Apr. 3	17. 41	Ham and cheese.
Apr. 10	16. 31	
Apr. 17	16. 65	Acetonitrile, 6.66 mgms., i. e., 0.4 mgm. per gm. mouse; died, 2½ hours.

B. INITIAL DIET: RICE. (v, 162.)

1905.	*Grams.*	
Apr. 3	15. 35	Diet of rice begun.
Apr. 10	13. 31	
Apr. 17	12. 05	Acetonitrile, 6.03 mgms., i. e., 0.5 mgm. per gm. mouse; survived.
Apr. 3	16. 05	Diet of rice begun.
Apr. 10	14. 25	
Apr. 17	11. 81	
Apr. 18	11. 31	Acetonitrile, 7.03 mgms., i. e., 0.6 mgm. per gm. mouse; survived.
Apr. 21	......	Diet changed to ham and cheese.
Apr. 26	14. 41	
May 3	17. 25	
May 4	17. 45	Acetonitrile, 6.98 mgms., i. e., 0.4 mgm. per gm. mouse; died, 5 hours. A few other mice of this group died after 14 to 17 days of the exclusive rice diet.

C. INITIAL DIET: OLIVE OIL ON OATS. (v, 160.)

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
Apr. 3	14. 21	Olive oil on oats.
Apr. 10	11. 11	
Apr. 17	10. 01	
Apr. 19	10. 22	Acetonitrile, 2.04 mgms. i. e., 0.2 mgm. per gm. mouse; survived.
Apr. 21	....	Diet changed to rice.
Apr. 26	10. 75	
May 3	9. 23	Acetonitrile, 5.54 mgms., i. e., 0.6 mgm. per gm. mouse; survived.
Apr. 3	12. 55	Oil on oats.
Apr. 10	11. 71	
Apr. 17	10. 51	
Apr. 20	11. 05	Acetonitrile, 2.76 mgms., i. e., 0.25 mgm. per gm. mouse; survived.
Apr. 21	....	Diet changed to rice.
Apr. 26	12. 55	
May 3	11. 51	
May 4	10. 72	Acetonitrile, 8.58 mgms., i. e., 0. 8 mgm. per gm. mouse; survived.
May 5	....	Diet changed to ham and cheese.
May 18	17. 92	
June 3	17. 82	
July 17	22. 05	Acetonitrile, 11.03 mgms., i. e., 0.5 mgm. per gm. mouse; died, 2½ hours.
Apr. 3	16. 26	Olive oil on oats.
Apr. 10	14. 71	
Apr. 18	13. 35	Acetonitrile, 4 mgms., i. e., 0.3 mgm. per gm. mouse; died. 4 to 6 hours.
Apr. 3	17. 10	Olive oil on oats.
Apr. 10	15. 85	
Apr. 17	13. 75	Acetonitrile, 5.5 mgms., i. e., 0.4 mgm. per gm. mouse; died, 4½ hours.

D. INITIAL DIET: BREAD AND OATS. (v, 164.)

1905.	*Grams.*	
Apr. 3	14. 56	Diet of bread and oats begun.
Apr. 10	14. 30	
Apr. 19	15. 31	Acetonitrile, 3.83 mgms., i. e., 0.25 mgm. per gm. mouse; survived.
Apr. 3	20. 85	Bread and oats.
Apr. 10	19. 82	
Apr. 17	19. 81	
Apr. 20	20. 06	Acetonitrile, 6.02 mgms., i. e., 0.3 mgm. per gm. mouse; died, 4 hours.
Apr. 3	12. 50	Bread and oats.
Apr. 10	12. 80	
Apr. 18	13. 01	Acetonitrile, 4.55 mgms., i. e., 0.35 mgm. per gm. mouse; died, 3 hours.

SUMMARY OF SERIES 28.—The effects upon the resistance to acetonitrile of the initial diets in the above experiments may be summarized as follows:

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
A. Ham and cheese	Increase 1.1 per cent......	0. 20	0. 30
B. Rice	Decrease 25 per cent........................	. 60	....
C. Oil and oats	Decrease 19 per cent........................	. 25	. 30
D. Bread (S) and oats	Increase 0.9 per cent........................	. 25	. 30

Thus the mice which had received the diet of rice were the most resistant; they also lost most in weight. The mice on the oil and oats diet lost almost as much in weight without any increase in their resistance. An examination of the above protocols show that the resistance to acetonitrile of mice which had been receiving a diet of ham and cheese or of olive oil and oats was increased when they were placed on a diet of rice, and that the resistance of mice which had been increased by a diet of rice was lowered by a diet of ham and cheese; the latter also caused a rapid increase in weight. The bread used in the above experiments was from the same bakery as that used in the previously described experiments in which a low degree of resistance resulted from a bread diet.

Results similar to the above were obtained in the following experiments:

Series 29.

A. INITIAL DIET: HAM AND CHEESE. (v, 158–159.)

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
May 27	15.61	Diet of ham and cheese begun.
June 10	15.55	
June 19	18.10	Acetonitrile, 3.8 mgms., i. e., 0.21 mgm. per gm. mouse; died, 1¾ hours.
May 5	15.25	Diet of ham and cheese begun.
May 19	16.05	
June 3	19.85	
June 19	18.72	Acetonitrile, 4.68 mgms., i. e., 0.25 mgm. per gm. mouse; died, 1½ hours.
May 26	18.15	Diet of ham and cheese begun.
June 3	19.05	
June 18	16.26	Acetonitrile, 4.88 mgms., i. e., 0.3 mgm. per gm. mouse; died, 1 hour.

B. INITIAL DIET: RICE. (v, 162.)

1905.	*Grams.*	
May 27	18.21	Diet of rice begun.
June 3	17.55	
June 14	14.91	
June 18	13.30	Acetonitrile, 9.31 mgms., i e., 0.7 mgm per gm. mouse; survived.
June 20	13.22	Diet changed to ham and cheese.
June 30	17.35	
July 3	19.22	Acetonitrile, 9.61 mgms., i. e., 0.5 mgm. per gm. mouse; died, 12 to 18 hours.

Series 30.

A. INITIAL DIET: RICE. (v, 166.)

1905.	*Grams.*	
June 21	20.15	Diet of rice begun.
June 30	18.95	
July 10	15.05	Acetonitrile, 10.54 mgms., i. e., 0. 7 mgm. per gm. mouse; survived.
June 21	21.35	Diet of rice begun.
June 30	18.35	
July 10	15.06	Acetonitrile, 12.05 mgms., i. e., 0.8 mgm. per gm. mouse; survived.
July 12	15.05	Diet changed to ham and cheese.
July 31	22.35	
Aug. 2	23.05	Acetonitrile, 11.53 mgms., i. e., 0.5 mgm. per gm. mouse; died, 2 hours.

B. INITIAL DIET: HAM AND CHEESE. (v, 165.)

1905.	*Grams.*	
June 21	19.15	Diet of ham and cheese begun.
July 6	18.05	
July 10	16.05	Acetonitrile, 3.21 mgms., i. e., 0.2 mgm. per gm. mouse; died, 2 hours.

These experiments also show that the change from a diet of rice to one of ham and cheese caused a marked increase in weight and a low resistance.

Series 31.

A. DIET: OATS AND WATER. (v, 30.)

Date.	Weight of mouse.	Remarks.
1904.	*Grams.*	
Nov. 11	19.51	Diet of oats and water begun.
Nov. 25	17.23	
Dec. 11	19.35	Acetonitrile, 6.77 mgms., i. e., 0.35 mgm. per gm. mouse; survived.
Nov. 8	21.86	Diet of oats and water begun.
Nov. 25	19.36	
Dec. 11	19.43	Acetonitrile, 7.38 mgms., i. e., 0.38 mgm. per gm. mouse; survived.
Nov. 8	22.35	Diet of oats and water begun.
Nov. 25	20.95	
Dec. 10	19.11	Acetonitrile, 7.64 mgms., i. e., 0.4 mgm. per gm. mouse; died, 3 to 9 hours.

B. DIET: OATS IN 20 PER CENT DEXTROSE. (v, 30.)

1904.	*Grams.*	
Nov. 8	21.56	Diet of oats and dextrose begun.
Nov. 25	20.85	
Dec. 10	20.02	Acetonitrile, 8 mgms., i. e., 0.4 mgm. per gm. mouse; survived.
Nov. 8	18.31	Diet of oats and dextrose begun.
Nov. 25	18.02	
Dec. 11	18.85	Acetonitrile, 8.48 mgms., i. e., 0.45 mgm. per gm. mouse; survived.
Dec. 12	....	Diet of oats and dextrose continued.
Dec. 25	17.71	Acetonitrile, 14.17 mgms., i. e., 0.8 mgm. per gm. mouse; survived.

C. DIET: AMERICAN CHEESE. (v, 31.)

1904.	*Grams.*	
Nov. 8	24.45	Diet of cheese begun.
Nov. 25	20.70	
Dec. 11	16.31	Acetonitrile, 5.71 mgms., i. e., 0.35 mgm. per gm. mouse; survived.
Dec. 12	....	Diet of cheese continued.
Dec. 23	13.31	Acetonitrile, 4.79 mgms., i. e., 0.36 mgm. per gm. mouse, died, 6 to 8 hours.
Nov. 8	25.85	Diet of cheese begun.
Nov. 25	24.35	
Dec. 13	19.22	Acetonitrile, 7.3 mgms., i. e., 0.38 mgm. per gm. mouse; died, 3¾ hours.
Nov. 8	23.45	Diet of cheese begun.
Nov. 25	19.52	
Dec. 10	17.10	Acetonitrile, 6.84 mgms., i. e., 0.4 mgm. per gm. mouse; died, 3 to 9 hours.

Summary of Series 31.—The above experiments may be summarized as follows:

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
A. Oats and water	Decrease 9 per cent	0.38	0.40
B. Oats and dextrose	No change	.55	...
C. Cheese	Decrease 24 per cent	.35	.38

Thus there was an increased resistance to acetonitrile without any increase in weight and a lowered resistance with a considerable loss of weight.

With a shorter period of feeding, the effect of dextrose in increasing the resistance of mice to acetonitrile was less marked, as is shown in the following:

Series 32.

A. DIET OF OATS AND WATER. (v, 170–171.)

[Dose in mgm. per gm. mouse.]

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
Jan. 9	18.21	Diet of oats and water begun.
Jan. 13	17.02	
Jan. 21	18.41	Acetonitrile, 6.44 mgms., i. e., 0.35 mgm. per gm. mouse; survived.
Jan. 9	14.51	Diet of oats and water begun.
Jan. 22	15.61	Acetonitrile, 6.24 mgms., i. e., 0.4 mgm. per gm. mouse; died, 17 hours.

B. DIET OF OATS IN 20 PER CENT DEXTROSE. (v, 32.)

1905.	*Grams.*	
Jan. 9	17.85	Diet of oats and dextrose begun.
Jan. 23	18.15	Acetonitrile, 7.26 mgms., i. e., 0.4 mgm. per gm. mouse; survived.
Jan. 9	18.07	Diet of oats and dextrose begun.
Jan. 21	19.15	Acetonitrile. 9.58 mgms.. i. e , 0.5 mgm. per gm. mouse; died, 8 hours.

Series 33. (Mar., 1906.) (v, 136.)

In the following experiments the effects of adding alcohol, dextrose and alcohol, and dextrose to the diet of oats and water was determined. A number of mice were placed upon these diets (with others on diets of oats and water and dried beef) and their resistance to acetonitrile tested after periods of 7, 11, and 14 weeks. As the experiments were numerous, only a summary of the results will be given:

A. AFTER 7 WEEKS.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
A. Oats and water	Increased 6.7 per cent	0.58	0.62
B. Oats in alcohol gradually increased from 5 to 50 per cent.	Decreased 8.7 per cent	.60	.62
C. Oats in dextrose solution increased from 5 to 20 per cent.	Increased 16.7 per cent	.50	.55
D. Oats in dextrose solution (increased from 5 to 20 per cent) and alcohol (increased from 5 to 50 per cent.)	Increased 6 per cent	1.20	1.30
E. Dried beef in water	Decreased 8.8 per cent	1.00	....

B. AFTER 11 WEEKS.

A. Oats and water	Increased 3.3 per cent	0.80	....
B. Oats in alcohol	Decreased 5.6 per cent	.30	0.35
C. Oats and dextrose	Increased 7 per cent	....	1.00
D. Oats and alcohol and dextrose	Decreased 2.7 per cent	.90	1.10
E. Dried beef in water	Decreased 6.5 per cent	1.20	....

C. AFTER 14 WEEKS.

Date.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
A. Oats and water	Decreased 3.3 per cent	0.90	1.10
B. Oats and alcohol	Decreased 20 per cent	.60	....
C. Oats, alcohol, and dextrose	Increased 2 per cent	1.10	1.10
D. Dried beef in water	Increased 2 per cent	1.00	1.20

Summary of Series 33.—In the above experiments there were, after 7 weeks, no marked differences in the susceptibility to acetonitrile of the mice upon the different diets except that the mice which had received both alcohol and dextrose, and those which had received beef, were much more resistant to this poison than were the others. After 11 weeks the characteristic effects of alcohol in diminishing the resistance of animals to acetonitrile and of oats in increasing it were manifest. The change in weight of the mice in the above experiments was, in most cases, so slight that not much importance is to be attached to it as a cause of the differences in the susceptibility.

That an exclusive diet of lean dried beef will lead to an increased resistance to acetonitrile, and that alcohol added to such diet will diminish the effect of the beef, is shown by the following experiments; the alcohol was slowly increased from 5 to 50 per cent.

Series 34. (Feb., 1906.) (v, 132.)

A. AFTER 3 TO 4 WEEKS.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
A. Oats and water	Decreased 2 per cent	0.50	0.60
B. Beef and water	Increased 15 per cent	.40	......
C. Beef and alcohol	Decreased 10 per cent	.35	......

B. AFTER 7 WEEKS.

A. Oats and water	Increased 6.7 per cent	0.58	0.62
B. Beef and water	Decreased 8.8 per cent	1.00	....
C. Beef and alcohol	Decreased 20 per cent	.50	.55

C. AFTER 11 WEEKS.

A. Oats and water	Increased 3.3 per cent	0.80	....
B. Beef and water	Increased 22 per cent	1.30	1.30

D. AFTER 14 WEEKS.

A. Beef and water	Increased 25 per cent	1.10	....

In the following experiments the diet consisted of "cakes" made by mixing varying amounts of other foodstuffs with ordinary "crackers" or "biscuits;" the controls were fed on "cakes" made from the latter only. The experiments, which were very numerous, are given in tabulated form. Only the largest nonfatal and the smallest fatal doses are recorded.

Series 35. (Oct., 1905.) (v, 176.)

The mice were fed on the diets named below for 12 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Cakes	Decreased 12 per cent	0.20	0.25
b. Lard, 1 part; cakes, 3 parts	Decreased 13 per cent	.15	.20
c. Corn starch, 1 part; cakes, 3 parts	Decreased 9 per cent	.15	.20
d. Casein, 1 part; cakes, 3 parts	Decreased 1 per cent	.18	.20
e. Dextrose, 1 part; cakes, 3 parts	Decreased 7 per cent	.25	.30

Series 36. (Oct., 1905.) (v, 178.)

The mice were fed on the diets named below for 13 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered	Died.
a. Cakes	Decreased 11 per cent	0.27	....
b. Lard, 2 parts; cakes, 2 parts	Decreased 28 per cent	....	0.15
c.[a] Casein, 2 parts; cakes, 2 parts	Decreased 18 per cent	.12	.15
d. Starch, 2 parts; cakes, 2 parts	Decreased 29 per cent	.20(?)	.30
e. Dextrose, 2 parts; cakes, 2 parts	Decreased 16 per cent	.35	.40

[a] Another mouse of this group died from 0.18 mgm. acetonitrile. A mouse which had recovered from 0.12 mgm. per gm. was placed for 2 weeks upon dextrose cakes; it recovered from 0.3 mgm. acetonitrile per gm., i. e., from a dose twice as large as the fatal dose for mice on casein.

Series 37. (Nov., 1905.) (v, 90.)

The mice were fed on the diets named below for 8 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 16 per cent	....	0.30
b. Lard and cakes, equal parts	Decreased 31 per cent	....	.12
c. Starch and cakes, equal parts	Decreased 31 per cent	....	.25
d. Casein and cakes, equal parts	Decreased 27 per cent	0.25	....
e. Dextrose and cakes, equal parts	Decreased 12 per cent	.35	....
f. Ham and cheese, equal parts	Increased 3.5 per cent	.15	....
g. Egg and cheese, equal parts	Decreased 1 per cent	.20	.30
h. Rice	Decreased 23 per cent	.65	.80

Some of the mice in this series which had acquired a high degree of resistance to acetonitrile as a result of the rice or dextrose diet were placed upon a diet of ham and cheese; after about 2 weeks they were again tested with acetonitrile. Their resistance was found to be markedly diminished, although their weight had increased and their general condition had apparently improved.

Summary of Series 35–37.—The addition of lard, in comparatively large proportion, to the diet of mice reduces their resistance to acetonitrile markedly; it also causes a marked loss of weight. The effect of corn starch is apparently the same; casein in large amounts caused a marked loss of weight, but its effect upon the resistance to acetonitrile was variable. Dextrose, which in these experiments caused a loss of weight (which was, however, rather less than that caused by the exclusive diet of cakes alone) caused a decided increase in the resistance. Rice, as usual, caused a marked loss of weight and also a marked increase in the resistance. There were, however, in the above experiments several cases in which a marked loss of weight was not accompanied by an increased resistance.

Several extensive series of experiments were performed in which small amounts of various foods, blood, various organs of animals, and certain other substances which may, at times at least, have some interest in connection with dietetic studies, were added to cakes and fed to mice. Many of these experiments were performed as controls to other experiments, and in this respect the negative results often obtained have a certain interest. They will be reported in as brief a form as possible.

Series 38. (Aug., 1905.) (v, 30.)

The mice were fed on the diets named below for about 4 weeks. Such expressions as "casein 0.3+4" indicate that 0.3 gm. casein was made into a cake with 4 gms. cracker dust.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Cakes	Decreased 28 per cent	0.20	......
b. Peptone, 0.3+4	Decreased 25 per cent	.20	0.30
c. Nutrose, 0.3+4	Decreased 5 per cent	.29	.30
d. Gelatine, 0.3+4	Decreased 32 per cent	.35	.45
e. Casein, 0.3+4	Decreased 25 per cent	.15	......

Series 39. (Oct., 1905.) (v, 86-87.)

The mice were fed on the following diets for about 1 week.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Increased 0.8 per cent	0.20	0.25
b. Parathyroids (beef), 0.2+4	Increased 9 per cent	.16	.15
c. Casein, 0.2+4	Increased 6 per cent	.22	.28
d. Peptone, 0.2+4	Increased 9 per cent	.25	....

Series 40. (July, 1905.) (v, 33.)

The mice were fed for about 1 week.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 9 per cent		0.15
b. Casein, 0.3+4	Increased 4 per cent	0.12	.15
c. Suprarenal, 0.3+4	Increased 21 per cent		.15
d. Thymus, 0.3+4	Increased 1 per cent	.18	.20
e. Nuclein (yeast), 0.3+4	Decreased 2 per cent	.20	.25
f. Gelatine, 0.3+4	Decreased 8.8 per cent	.23	.30

Series 41. (Dec., 1906.) (v, 130.)

The mice were fed for about 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Increased 0.5 per cent	0.55	0.55
b. Pancreas (desiccated), 0.3+4	Increased 13 per cent		.35
c. Spleen (desiccated), 0.3+4	Increased 9 per cent	.35	.50
d. Tartaric acid, 0.1+4	Decreased 4 per cent	.45	.65
e. Brain (desiccated), 0.3+4	Increased 6.8 per cent	.65	.80
f. Parotid (desiccated), 0.3+4	Decreased 0.9 per cent	.60	.80
g. Mammary gland (desiccated), 0.3+4	Increased 12 per cent	2.00	
h. Prostate (desiccated), 0.3+4	Increased 9 per cent	2.00	
i. Testes (desiccated), 0.3+4	Increased 8.5 per cent	2.00	

Some of the mice of the groups *g*, *h*, *i*, of the above table were placed upon plain cakes and their resistance to acetonitrile again tested after a week. Mice which had received the mammary gland (and which had recovered from 2 mgms. per gm.) now died from 0.4, 0.5, and 0.6 mgm. acetonitrile; of mice which had received the prostate, one recovered from 0.4 mgm. and one died from 0.6 mgm. per gm. mouse, whereas of those which had received the testes, two recovered from 0.7 and 0.9 mgm. These results show the brief duration of the increased resistance caused by prostate and mammary gland.

Series 42. (Sept., 1905.) (v, 78.)

The mice were fed on the following diets for 7 to 8 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Increased 7 per cent	0.23	0.25
b. Parathyroids (beef), 0.05+4	Increased 10 per cent	.10	.20
c. Ovaries, 0.3+4	Increased 3 per cent	.23	.30

Series 43. (Sept., 1905.) (vi, 86.)

The mice were fed as below for about 1 week.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 10 per cent	0.20	0.22
b. Parathyroids (beef) 0.2+4	Decreased 7 per cent	.08	.10

Series 44. (Dec., 1906.) (vii, 142.)

The mice were fed as below for 9 to 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Diet.
a. Cakes	Decreased 8 per cent	0.50	0.65
b. Mammary gland, 0.1+4	Increased 1 per cent	1.30	1.40
c. Testes, 0.1+4	Increased 2 per cent	1.40	1.50
d. Prostate, 0.1+4	No change	3.00	....

Three mice of group *d* were given a diet of plain cakes for a week and again tested with acetonitrile; 1 recovered from 0.5 mgm., 1 died from 0.7 mgm. and another from 0.8 mgm. per gm.; thus the high resistance caused by the prostate was of short duration.

Series 45. (May, 1907.) (vii, 126.)

The mice were fed as below for 12 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 5.5 per cent	0.22	0.23
b. Mammary gland, 0.05+4	Increased 3 per cent	.26	.30
c. Ovaries, 0.1+4	Increased 8 per cent	.35	.40
d. Corpus luteum (desiccated), 0.1+4	Decreased 3 per cent	....	.28

Series 46. (Dec., 1906.) (vii, 144.)

The mice were fed as below for 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	No change	....	0.40
b. Pancreas, 0.1+4	Increased 10 per cent	0.44	.55
c. Tamarinds (pulp) 0.3+4	Increased 5 per cent	.70	.85
d. Ovaries, 0.3+4	Increased 10 per cent	1.10	1.60

Series 47. (May, 1907.) (vii, 122.)

The mice were fed as below for 18 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 7 per cent		0.28
b. Prostate, 0.05+4	Decreased 15 per cent	1.00	1.40

Series 48. (Jan., 1907.) (vii, 174.)

The mice were fed as below for 13 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 5 per cent	0.80	0.90
b. Guinea-pig embryos (desiccated), 0.3+4	Increased 4 per cent	.60	.70
c. Prostate, 0.01+4	Increased 3 per cent		.85

Series 49. (June, 1907.) (vii, 82.)

The mice were fed as below for 11 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 5 per cent	0.31	0.32
b. Milk sugar, 0.3+4	Decreased 12 per cent	.27	.30

Series 50. (Dec., 1906.) (vii, 140.)

The mice were fed as below for 12 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 2 per cent	0.70	0.75
b. Umbilical cord (desiccated), 0.1+4	No change		.90
c. Placenta (desiccated), 0.3+4	Increased 1.5 per cent	.70	.85

Series 51. (Dec., 1906.) (vii, 135.)

The mice were fed as below for 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Increased 1 per cent	0.45	0.48
b. Saccharin, 0.1+4	Decreased 0.5 per cent	.55	.48
c. Carcinoma of liver, 0.2+4	Decreased 2 per cent	.90	1.10
d. Egg albumen (dried), 0.4+4	Increased 3 per cent	.45	.60
e. Ox gall (purified), 0.2+4	Decreased 20 per cent	.60	.80

Series 52. (May, 1906.) (vi, 190.)

The mice were fed as below for 16 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Oats and water	Increased 7 per cent	0.45	0.50
b. Oats and 5 per cent glycerin	Decreased 5.5 per cent	.37	.40

Series 53. (Nov., 1906.) (vi, 179.)

The mice were fed as below for 11 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	No change	0.70	0.80
b. Kidney (dog), 0.2+4	Decreased 1.5 per cent	.90	1.00

Series 54. (Mar., 1906.) (vi, 180.)

The diet was continued for 23 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 17 per cent	0.21	0.25
b. Meat extract (Liebig) 0.2+4	Decreased 19 per cent	.27	.30

Series 55. (Nov., 1906.) (vi, 156.)

The mice were fed as below for 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 13 per cent		0.50
b. Chocolate, 0.3+4	Decreased 5 per cent	0.40	1.00
c. Liver (human), 0.2+4	Decreased 11 per cent	.75	.80
d. Egg albumen (dry), 0.2+4	Decreased 9 per cent	1.00	1.30

Series 56. (Nov., 1906.) (vi, 194.)

The mice were fed as follows for 11 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Increased 1 per cent	0.15	0.20
b. Sodium salicylate, 0.02+4	Increased 4.6 per cent	.24	.25
c. Sodium benzoate, 0.02+4	Increased 4 per cent	.30	.40
d. Pepsin, 0.05+4	Increased 1 per cent	.30	.40
e. Pancreatin, 0.05+4	Increased 7 per cent	.30	.60

Series 57. (Nov., 1905.) (vi, 140.)

The mice were fed as below for 7 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 1 per cent	0.30	0.35
b. Pituitary body, 0.1+4	Increased 15 per cent	.20	.25

Series 58. (Nov., 1906.) (vi, 171.)

The mice were fed as below for 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 1 per cent	0.45	0.55
b. Prunes, 0.2+4	Increased 6 per cent	.30	.50
c. Tartaric acid, 0.2+4	Decreased 17 per cent	.75	....

Series 59. (Feb., 1906.) (vi, 158.)

The mice were fed as below for 13 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Increased 2 per cent	....	0.30
b. Submaxillary gland (ox), 0.5+4 (desiccated).	Increased 4 per cent	0.25	.33

Series 60. (Aug., 1905.) (vi, 36.)

The mice were fed as below for 13 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Not recorded	0.20	0.24
b. Suprarenal gland, 0.1+4	Decreased 3 per cent	.10	.15
c. Nucleic acid, 0.1+4	Decreased 13 per cent	.18	.20
d. Thymus, 0.1+4	Decreased 16 per cent	.18	.20

Series 61. (Aug., 1905.) (v, 81 and 49.)

The mice were fed as below for 29 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Oats in water	Increased 7 per cent	0.50	0.65
b. Oats in 0.2 per cent hydrochloric acid	Decreased 10 per cent	.70	.90

Series 62. (Jan., 1905.) (iv, 170 and v, 50.)

The mice were fed as below for 2 weeks.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Oats in water	Increased 3 per cent	0.35	0.40
b. Oats in 0.5 and then 0.25 per cent hydrochloric acid.	Decreased 12 per cent	.60	....

Series 63. (Mar., 1908.) (x, 152.)

The mice were fed as below for 11 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 14 per cent	0.40	0.42
b. Ammonium acetate, 0.1+4	Decreased 12 per cent	.65	.75
c. Sulphur, 0.01+4	Decreased 9 per cent	3.90	4.00

Series 64. (Jan., 1908.) (x, 70.)

The mice were fed as below for 11 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Increased 3 per cent	0.37	0.38
b. Magnesium chloride, 0.05+4	Increased 6 per cent	.40	.50
c. Caffeine 0.03+4	Increased 1 per cent	.40	.60
d. Calcium chloride, 0.05+4	Increased 0.5 per cent	1.10	1.2

Series 65. (Oct., 1908.) (xi, 74.)

The mice were fed as below for 8 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 4 per cent	0.36	0.37
b. Cystin, 0.05+4	Decreased 6 per cent	.54	.60

Series 66. (Mar., 1908.) (x, 114.)

The mice were fed as below for 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Increased 2 per cent	0.48	0.50
b. Sulphur 0.05+4	Decreased 8 per cent	3.48	3.80

Series 67. (Jan., 1908.) (x, 74.)

The mice were fed as below for 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	No change	0.70	0.80
b. Borax, 0.05+4	Decreased 7 per cent		.40
c. Saponin, 0.01+4	Decreased 4 per cent	.53	.60

Series 68. (Sept., 1905.) (vi, 29.)

The mice were fed as below for 18 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 8 per cent	0.3	0.33
b. Copper acetate, 0.0056+4	Decreased 7 per cent		.40

Series 69. (Dec., 1905. (vi, 90.)

The mice were fed as below for 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 13 per cent	0.35	0.40
b. Taurin, 0.3+4	Decreased 9 per cent	.50	.45
c. Cystin, 0.3+4	Decreased 30 per cent	.75	.80
d. Sulphur, 0.1+4	Decreased 23 per cent	2.00	3.00

Several mice of groups *c* and *d* were placed upon plain cakes; there as still an increased resistance to acetonitril three weeks later.

Series 70. (Nov., 1907.) (x, 78.)

A few c. c. of blood were obtained from a woman who had died after an operation for exophthalmic goitre; there was only enough for experiments upon two mice. Control experiments were made with normal human and rabbit blood.

The mice were fed as below for 11 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 2 per cent		0.25
b. Rabbit blood, 0.5 c. c.+4	Decreased 2 per cent	0.25	.30
c. Normal human blood, 0.5 c. c.+4	Decreased 4 per cent	.26	.30
d. Exophthalmic goitre blood 0.5 c. c.+4	Decreased 3 per cent		.35

Series 71. (May, 1908.) (x, 184.)

A small amount of blood was obtained from a mild case of exophthalmic goitre and fed as below for 9 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 1 per cent	0.25	0.30
b. Exophthalmic goitre blood, 1 c. c.+4	Decreased 8 per cent	.30	.33
c. Normal human blood, 1 c. c.+4	Decreased 12 per cent	.33	.35

Series 72. (June, 1907.) (vii, 34.)

The "exophthalmic goitre blood" of this and the next series was obtained at autopsy from a well-marked case of this disease. The results of these experiments have been described in more detail elsewhere.[a]

The mice were fed as below for 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Increased 3.5 per cent	0.38	0.39
b. Human blood (placenta), 1 c. c.+4	Increased 5.6 per cent	.37	.39
c. Exophthalmic goitre blood, 1 c. c.+4	Decreased 2.3 per cent	.70	.75

Series 73. (June, 1907.) (vii, 67.)

The mice were fed as below for 11 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 6.2 per cent	0.31	0.32
b. Exophthalmic goitre blood, 1 c. c.+4	Decreased 4.9 per cent	.45	.48

Series 74. (Jan., 1907.) (vii, 174.)

Two guinea pigs were fed for 5 days in succession 0.6 gm. desiccated thyroid; they were then bled to death and their blood used for the following experiments. Although these guinea pigs were deeply intoxicated with the thyroid, their blood gave no physiological test for thyroid.[b] (That blood does not interfere with the physiological test for thyroid is shown by experiments in which small amounts (0.1 mgm., for example) of thyroid were added to blood and fed to mice; the latter showed a markedly increased resistance to acetonitrile).

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[a] The Probable Demonstration of Thyroid Secretion in the Blood in Exophthalmic Goitre, Journ. Amer. Med. Assoc., 1907, 49, p. 240.

[b] The possible bearing of this upon the relation of hyperthyroidism to exophthalmic goitre was discussed in the paper referred to above.

The mice were fed as below for 12 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 5.2 per cent	0.80	0.90
b. Blood (dried) from thyroid-fed guinea pigs, 0.3 gm.+4.	Increased 4.1 per cent	.60	.85
c. Normal guinea pig blood (dried), 0.3 gm. +4.	Increased 5 per cent	....	.85

Series 75. (July, 1905.) (vi, 48.)

The mice were fed as below for 7 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 5.4 per cent	0.24	0.30
b. Peptone, 0.2+4	Decreased 10.6 per cent	.15	.17
c. Sheeps' blood (dried), 0.2+4	Decreased 17 per cent	.17	.20
d. Thyreoidectin,[a] 0.2+4	Decreased 7.5 per cent	....	.20

[a] See New and Non-official Remedies, 1910, p. 32.

Series 76. (June, 1905.) (v, 128.)

The mice were fed as below for 12 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Increased 0.6 per cent	0.30	0.32
b. Thyreoidectin, 0.3+4	Decreased 13.7 per cent	.08	.15

Series 77. (Jan., 1907.) (vii, 193.)

The mice were fed as below for 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Increased 8.1 per cent	0.37	0.40
b. Normal horse serum (dry), 0.3+4	Increased 10 per cent	.32	.37
c. Yeast, 0.3+4.	Increased 24 per cent	....	.35
d. Antidiphtheritic serum (dry), 0.3+4.	Increased 10 per cent	....	.75
e. Caffeine	Decreased 2.4 per cent	.90	....

Series 78. (July, 1905.) (vi, 34.)

The mice were fed as below for 12 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
a. Cakes	Decreased 19.5 per cent	....	0.30
b. Peptone, 0.3+4	Decreased 22 per cent	0.12	.15
c. Thyreoidectin, 0.3+4	Decreased 19 per cent	.20	.30

Series 79. (Feb., 1910.) (xii, 16.)

Many of the above experiments having shown that several fats and oils increase the susceptibility of mice to acetonitrile, the following experiments were made with corn meal from which the oil had been extracted by ether.

The mice were fed as below for about 7 weeks.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Recovered.	Died.
Corn meal	Increased 15.86 per cent (from 11.91 to 13.8 gms.)	1.40	1.50
Extracted corn meal	Increased 9.02 per cent (from 11.41 to 12.44 gms.)	2.00	2.10

Thus the resistance of the mice which had received the fat-free corn meal was distinctly greater than that of those which had received the fat-containing meal.

SUMMARY OF ABOVE RESULTS.

In many cases the experiments of the above series were not sufficiently numerous to permit of definite conclusions being drawn. There was also no constant standard of comparison, but it is not difficult in most cases to determine, in a general way, whether the resistance of mice to acetonitrile caused by a certain diet should be classed as "high," or "low," or "medium."

Cakes.—A larger number of experiments were performed with cakes than with any other single diet. An examination of the above tables shows clearly that the resistance of mice which had received an exclusive diet of cakes should be classed as "medium;" the fatal dose varied from about 0.22 mgm. to about 0.64 mgm. per gm. mouse. The most important factor in this variation is undoubtedly the season. This is clearly shown by the following table, which gives the average fatal dose for several months; the fatal dose for mice which had received a diet of bread [a] and oats is given for comparison.

	Cakes.	Bread and oats.		Cakes.	Bread and oats.
January	0.64	0.56	July	0.25	0.31
February	.30	.35	August	.22	.18
March	.38	.58	September	.24	.25
April		.53	October	.27	.39
May	.25	.30	November	.41	.45
June	.33	.28	December	.52	.55

Inasmuch as in many cases the diet of cakes was continued for but a short period and the mice had received previously various other diets, it is not improbable that the latter had in certain cases exerted an influence upon the effects of the former.

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[a] The bread used in all of the experiments was of the brand described as "S;" it always caused a relatively low degree of resistance.

Oats and oatmeal.—Mice kept on a diet of oats and oatmeal were usually very resistant to acetonitrile; in most series of experiments they were more resistant than the mice upon any other diet. The length of time during which they received this diet was a very important factor in the degree of resistance attained; when transferred from a mixed diet to one of oats the increased resistance came slowly and, on the other hand, when mice which had become resistant on a diet of oats were placed on other diets the resistance disappeared slowly (cf. series 13).

With diets of barley, rye, and wheat the resistance was less (in the order named) than that with oats; hempseed also led to a low degree of resistance as compared with oats; thus in series 20 the fatal dose of acetonitrile was twice as large for the oats-fed as for the hempseed-fed mice. The effect of maize will be discussed later.

Bread.—Some of the most remarkable results obtained were with bread. The bread was obtained from two sources designated "C" and "S," respectively. In both cases it was sold as ordinary white wheat bread; the dealer supplying it considered the one the equivalent of the other. Yet mice receiving an exclusive diet of "C" were invariably much more (as much as four times as) resistant to acetonitrile than were those fed upon "S." This difference may have been due to the two brands containing different percentages of fat, or sugar, or milk, or salt, or to the kind of flour used, or to some special treatment of the latter.[a]

Whatever the explanation, it is evident that for careful dietary studies the exact composition of the bread should be known; it also seems desirable for the words "white wheat bread" to mean something more definite than they do at present. It is not entirely improbable that in certain cases of sickness different kinds of bread would have different effects analogous to those found in experiments on lower animals.

Maize.—An exclusive diet of corn meal usually caused a loss of weight and generally a high degree of resistance to acetonitrile. Corn starch added to cakes (1 or 2 to 2) caused a marked loss of weight and a low degree of resistance.

Milk.—An exclusive diet of milk led to a low degree of resistance; this was true both of raw and boiled milk. In two cases the resistance was less with the raw, in one with the boiled milk. In the latter case there had been a much more rapid increase in weight with the boiled than with raw milk. Series 13 shows how marked and rapid may be the lowering of resistance when mice are transferred from various diets to one of milk.

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[a] Doctor Hale found samples of the bread used to each contain "nitrite-reacting material" equivalent to about 0.3 per 1,000,000 sodium nitrite.

Milk added to oatmeal or corn meal led to a lower degree of resistance (with two exceptions) than did these alone; boiled milk seemed more effective than did raw milk. There was a greater increase in weight on the combined diet than on either alone. In two series (14 and 15) the resistance of mice was greater upon a diet of oatmeal and boiled milk than upon a diet of oatmeal alone.

A combined diet of bread and milk did not have an effect different from that of bread alone.

These results show how many factors are involved and what great variations in the resistance are caused by comparatively slight changes in the diet. The fact that mice are so very sensitive to such changes, however, increases the probability that this method may at least suggest promising lines of investigation. Thus the fact that at times boiled milk leads to a diminished resistance may be found to be due to a change in the sulphur content of such milk or to the sulphur compounds having undergone some change during the heating. It is well known that traces of sulphur are expelled from milk on boiling and it will be shown later that sulphur in some forms of combination is very effective in protecting animals against acetonitrile whereas in other forms it is inert in this regard. Differences in sulphur content or changes in its form of combination too small to be clearly detected by ordinary chemical means might be thus detected physiologically; and if a certain form of sulphur is an important factor in the resistance of animals to acetonitrile it is probable that it is important in relation to growth or resistance to other forms of intoxication. The fact that boiled milk, as compared with raw milk, does not invariably cause a diminished resistance may be due to there being compensating factors involved. Thus it is stated that boiling leads to a partial decomposition of the lecithin contained in milk. There are reasons for believing that lecithin, either directly or indirectly, causes an increased susceptibility in mice to acetonitrile. If such a decomposition occurred it might offset other effects of heat. However, the only value these results have at present is that they are suggestive of new lines of work.

Cheese led to a distinctly low degree of resistance whether (as in series 17) there was an increase in the weight of the mice or (as in series 31) a marked decrease.

Casein fed in cakes in large amounts distinctly lowered the resistance as compared with cakes.

Ham.—An exclusive diet of lean ham caused a low degree of resistance in the single experiment in which it was fed alone (17). A mixture of ham and cheese had a similar effect; this was true whether there had been a decrease or an increase in the weight of the mice. The experiments of series 28, 29, and 30 afford some rather striking illustrations of how the resistance of mice could be varied by changing from a diet of rice (which causes a high degree of resistance) to one of ham and cheese and vice versa.

Beef.—A diet of lean dried beef led to a marked increase in the resistance to acetonitrile; the addition of alcohol to the beef led to the opposite result, i. e., it lowered the resistance by about one-half. The beef caused an increase in weight; beef and alcohol caused a decrease. This is another illustration of the frequent independence of change in weight and changes in susceptibility. *Witte's peptone* added to cakes increased the susceptibility very distinctly. *Liebig's Extract* added to cakes had no distinct effect in the single experiment in which this was tested. *Gelatin* increased the resistance in two cases.

Liver.—An exclusive diet of liver led to a very great increase in the resistance to acetonitrile; this was greater than was the case with any other article of food (series 17). The addition of comparatively small amounts of dried liver to cakes (0.2 gm. to 4 gms. cakes) also increased the resistance as compared with cakes alone. Egg yolk diminished the resistance to acetonitrile; this effect was largely counteracted by the addition of liver. The diet of egg yolk and liver caused a marked increase in weight, whereas that of liver alone has, as a rule, but a slight effect. *Carcinoma* of the liver caused in one case a marked increase in resistance. *Purified ox gall* (U. S. P.) added to cakes (0.2 gm. to 4 gms.) caused a marked loss of weight and a slight increase in resistance. *Taurin* (0.3 + 4) caused a slight loss of weight and a slight increase in resistance.

Kidney.—An exclusive diet of kidney led to an increase in weight and a marked increase in resistance. The addition of a small amount of dried kidney (0.2 gm. to 4 gms. cakes) caused a slight increase as compared with a diet of cakes alone.

Brain.—Mice died in a few days upon an exclusive diet of brain. When fed with oatmeal (equal parts by weight) the resistance was less than when oatmeal alone was fed; there was a greater increase in weight in the former case. When dessicated brain was added to cakes (0.3 gm. to 4 gms.) there was a greater increase in weight and a greater degree of resistance than when cakes alone were fed.

Egg.—*Egg yolk* caused a very great diminution of resistance and a marked increase in weight in series 17 and 18; the entire egg had an equally great effect in series 18. Young mice were used in both of these series. With somewhat older mice there was a less marked increase in weight and a rather high degree of resistance. A diet of equal parts of egg yolk and liver caused a marked increase in weight and a degree of resistance intermediate between that caused by liver alone and egg yolk alone; it was nearer the latter than the former. Mice died in a very few days from an exclusive diet of egg white. Added to bread and liver in equal parts, it diminished the resistance as compared with bread or liver alone; in one case, when added to oatmeal, it seemed to increase the resistance as compared with oatmeal.

Olive oil.—Olive oil added to a diet of bread or of oats invariably caused a marked loss of weight and a diminution of resistance; the effect was more marked with bread than with oats, i. e., the latter seemed to counteract to some extent the effect of the oil. *Cotton-seed oil and corn oil* caused a still greater loss of weight and an equally great diminution of resistance. *Cod-liver oil* caused a less marked loss of weight and a less marked diminution of resistance. *Lard* had a similar effect. Glycerin caused a slight loss of weight and a slightly diminished resistance.

Dextrose.—Dextrose added to a diet of cakes or of oats caused an increased resistance; this developed rather slowly. It frequently caused an increase in weight, especially when fed with oats.

Rice invariably caused a marked loss of weight and a marked increase in resistance. In several experiments rice-fed mice which had recovered from a large dose of the nitrile were placed upon a diet of ham and cheese; their weight rapidly increased and they were also less resistant to the nitrile. Partial inanition may be in part responsible for the high degree of resistance, but corn starch added to cakes caused a loss of weight but lowered resistance.

Potatoes.—An exclusive diet of potatoes caused in the only experiment with it a loss of weight but a very high degree of resistance.

Beans ("navy," boiled) caused in one experiment a very slight loss of weight and comparatively low degree of resistance; an exclusive diet of *peanuts* in the same series caused a somewhat greater loss of weight and a high degree of resistance.

Tamarinds and *tartaric acid* added to cakes in small amounts increased the resistance somewhat; in one case the tartaric acid caused a marked loss of weight and a very considerable increase in resistance. *Prunes* in one experiment had no effect. *Hydrochloric acid* (0.2 per cent on oats) caused in two cases a loss of weight and increased resistance.

Blood.—Normal human and rabbit blood (fresh) increased the resistance slightly; dried sheep blood diminished it slightly. *Thyreoidectin* (the dried blood of sheep from which the thyroids had been removed) in two cases diminished the resistance; in one case the diminution was marked. Normal horse serum and antidiphtheritic and antitetanic sera had no distinct effect in the few experiments in which they were tried. Of more interest are the experiments in which the blood from cases of exophthalmic goiter was fed. In two series such blood (from a severe case) caused a marked degree of resistance; in another series with blood from a mild case the effect was not greater than with normal human blood.

Prostate (desiccated) added in small amounts (0.05–0.3 to 4 gms.) caused a marked increase in the resistance;[a] *testes* had a similar effect; *ovaries* also had a similar but less marked effect; *mammary gland* also increased the resistance markedly.[b] *Thymus* and *submaxillary* had no distinct effect. *Pituitary suprarenal* and *spleen* each in one case seemed to diminish the resistance; *parathyroid* in the three cases in which it was fed distinctly diminished the resistance. *Placenta* and *umbilical cord* had no effect; guinea pig embryos seemed, in one experiment, to slightly diminish the resistance. *Pancreatin* and *pepsin* seemed to increase the resistance. *Yeast* (0.3 to 4 gms. cakes) caused a marked increase in weight and a slightly diminished resistance.

Sulphur even in small amounts invariably caused a marked increase in resistance.[c] *Cystin* caused a distinct but not very marked increase in the resistance; the effect of *taurin* was still less. *Caffeine* in two cases caused an increased resistance without change in weight. *Sodium salicylate, sodium benzoate,* and *ammonium acetate* each in one case increased the resistance slightly.

DISCUSSION.

The above experiments show that mice fed upon articles of food such as enter largely into the daily diet of man vary greatly in their resistance to a definite poison. In extreme cases mice after having been fed upon certain diets may recover from forty times the dose of acetonitrile fatal to mice kept upon other diets. It is, moreover, possible to alter the resistance of these animals at will and to overcome the effects of one diet by combining it with another. In some cases the changes may be brought about in a very short time, in others they are produced more slowly. Many questions are suggested by these results; an answer can be given to but a very few of them.

Does the diet influence the resistance to hydrocyanic acid? The increased resistance to acetonitrile caused by certain diets could be attributed to one of two factors, (1) an increased resistance to the hydrocyanic acid liberated from the nitrile, or (2) a diminished formation of hydrocyanic acid. Some evidence for the second supposition will be presented later; the following experiments indicate that some of the diets which cause an increased resistance to acetonitrile do not cause a similar increased resistance to hydrocyanic acid. Experiments (series 8) have already been cited to show that a restricted diet does not cause an increased resistance to another cyanogen compound (nitroprussiate of soda).

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[a] The effect of prostate upon the resistance of mice to acetonitrile is the same as, but much less marked than, that of thyroid. Prostate increases the susceptibility of rats and guinea pigs to this poison; having the same effect upon these animals as thyroid.

[b] Several clinicians have called attention to a relation between the thyroid and mammary glands. Thus Sainton and Fernet (Progrès Médical, 1908, p. 279) state that the latter are often found hypertrophied in myxœdema and atrophied in Grave's disease; Afert (Sem. méd., 1908, p. 71) and others report myxœdema as occurring after removal of the breasts.

[c] Sulphur likewise increases the resistance of rats and guinea pigs to acetonitrile, the effect being due to the neutralization, through the formation of sulphocyanic acid, of the hydrocyanic acid formed from the nitrile.

It was shown above that a diet of ham and cheese diminishes the resistance of mice to acetonitrile, whereas a diet of rice increases such resistance. The following experiments show that a similar relation does not hold as regards hydrocyanic acid.

Series 80. (**May, 1905.**) (**v, 190.**)

A. HAM AND CHEESE.

Date.	Weight of mouse.	Remarks.
1905.	*Grams.*	
May 3	23.21	Diet of ham and cheese begun.
May 12	22.32	
May 19	23.45	
May 26	23.15	Hydrocyanic acid, 0.0695 mgm., i. e., 0.003 mgm. per gm. mouse; survived.
May 5	17.41	Diet of ham and cheese begun.
May 12	17.62	
May 19	16.85	
May 26	18.45	Hydrocyanic acid, 0.059 mgm., i. e., 0.0032 mgm. per gm. mouse; survived.
May 26	17.15	
May 27	....	Diet of ham and cheese begun.
June 3	14.71	
June 10	14.12	
June 19	15.55	Hydrocyanic acid, 0.0498 mgm., i. e., 0.0032 mgm. per gm. mouse; survived.
May 5	14.81	Diet of ham and cheese begun.
May 12	13.52	
May 19	13.75	
May 26	12.25	Hydrocyanic acid, 0.0429 mgm., i. e., 0.0035 mgm. per gm. mouse; died, 15 minutes.

B. RICE.

1905.	*Grams.*	
May 5	20.51	Diet of rice begun.
May 12	18.92	
May 19	17.05	
May 26	14.31	Hydrocyanic acid, 0.0372 mgm., i. e., 0.0026 mgm. per gm. mouse; died, 1 hour 40 minutes.
May 5	15.41	Diet of rice begun.
May 12	12.65	
May 19	13.01	
May 26	11.41	Hydrocyanic acid, 0.0343 mgm., i. e., 0.003 mgm. per gm. mouse; died, 1 hour.
May 5	15.41	Diet of rice begun.
May 12	12.95	
May 19	12.25	
May 26	10.71	Hydrocyanic acid, 0.0375 mgm., i. e., 0.0035 mgm. per gm. mouse; died, 45 minutes.

Thus the mice which had been fed upon rice were more susceptible to hydrocyanic acid than were those which had had a diet of ham and cheese; i. e., the effect was just the opposite to that obtained with acetonitrile.

In previous experiments I found that whereas mice to which thyroid had been fed showed a very marked resistance to acetonitrile they were slightly more susceptible to hydrocyanic acid and nitroprussiate of soda.[a] The thyroid had caused in these experiments a loss of weight and I am inclined to attribute the increased susceptibility to these poisons to a lowering of "general resistance" rather than to any specific effect; the same probably holds true for the increased susceptibility to hydrocyanic acid which the mice which had been on a rice diet showed in these experiments.

These experiments afford support to the view that the protective action of a rice diet depends upon its preventing, in some way, the liberation of hydrocyanic acid from acetonitrile and not upon its exerting a favorable influence upon the neutralization of hydrocyanic acid; for in the latter case it would be expected that such a diet would reduce the susceptibility of mice to hydrocyanic acid itself, which is not the case.

Relation of changes of weight and rate of growth to resistance to acetonitrile.—It was shown in Part I that a restricted diet, which is accompanied by a marked loss of weight, increases the resistance of mice (and guinea pigs) to acetonitrile and the question was raised whether a marked loss of weight caused by other agencies might not be associated, causally, with an increased resistance to this poison. It also seemed probable, on theoretical grounds, that a diet which caused rapid growth and an increase in weight would, as a result of the increased metabolic activities, cause a more rapid decomposition of the acetonitrile molecule with a corresponding increase in the amount of hydrocyanic acid produced; hence mice (and other animals) receiving such a diet would be expected to be more susceptible to acetonitrile than those receiving diets which did not cause such a rapid growth. Several of the above experiments appear, at first sight, to support such a hypothesis. Thus the effect of an exclusive diet of rice was strikingly similar to that of a very restricted diet; there was a marked loss of weight and a high degree of resistance. When the rice diet was followed by one of ham and cheese there was, as a rule, an increase in weight and a marked diminution of the resistance to acetonitrile. In many other cases also the highest resistance occurred with the diets which caused but slight increases in weight and the lowest resistance with diets which caused the most rapid growth. The latter was especially marked in the case of a diet of eggs; the latter sometimes caused an increase of 40 per cent in weight in the course of a few weeks and the development of a very great susceptibility to acetonitrile. In certain cases in which the growth of the mice on this diet had been less marked, the susceptibility to the poison was also less. Moreover, the more rapid growth caused by adding milk to oatmeal or corn meal was usually accompanied by an increased susceptibility as compared with the resistance caused by the oatmeal or corn meal alone. In two of the longer series of experiments described above (17 and 18) there was a close parallelism between the degree of susceptibility and the rate of growth; in another series (13), however, there seemed to be no relation between the two.[a]

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[a] Jour. Biolog. Chem., 1905, i, p. 33.

On the other hand, there were many cases in which a loss of weight was not accompanied by an increase in resistance and others in which it was accompanied by a distinctly increased susceptibility. The latter was invariably the case, for example, when oils or fats were added to the diet. There were also many cases in which there was a rapid growth accompanied by an increased resistance. Thus although the addition of milk to oatmeal or corn meal increased the growth and lowered the resistance as compared with the effects of oatmeal or corn meal alone, it increased both the rate of growth and resistance as compared with an exclusive diet of milk. Similarly in series 18 a combination of egg yolk and liver led to as rapid a growth as did a diet of egg yolk alone, but the fatal dose of acetonitrile was three times as great in the former as in the latter case.

Notwithstanding these facts, I believe that in some cases there is a close, although perhaps indirect, relation between the influence of a diet upon growth and resistance to acetonitrile; this will be discussed in connection with the effect of diets upon the activity of the thyroid.

Relation to fats, proteins, and carbohydrates.—In order to determine whether there is a definite relation between resistance to acetonitrile and the proportion of fat, protein, and carbohydrates much greater attention to the composition of the diets would be necessary than was given to it in these experiments; the latter do, however, suggest certain general conclusions. Thus fats (lard, olive, cotton seed, corn, and cod liver oils) always led to a low degree of resistance; this was also the case in experiments upon rats. The effect of oil in corn meal is shown by the experiments in which extracted and unextracted meal was fed to mice; the latter caused a lower resistance than did the former. The low degree of resistance caused in mice by diets of milk, eggs, and cheese may also be connected in part with the large fat content of these diets; the same may also be true, to a less extent, for "cakes" which contain considerable (about 8 per cent) of fat.

Rice, which is very poor in fat, caused a high degree of resistance, but so did oats and oatmeal which are fairly rich in fats. Wheat and most kinds of wheat bread contain but little fat, but as a rule they cause a low degree of resistance.

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[a] Series 17 and 18 were performed in the summer, series 13 in the winter; whether season has an influence remains to be determined.

Dextrose increased the resistance of mice to acetonitrile.[a] Thus fats and at least some carbohydrates have opposite effects, thereby affording another illustration of the nonequivalence, in certain respects, of these food stuffs.[b]

Certain proteins (casein, peptones, and egg albumin) added to other foods lowered the resistance.

Before leaving this subject attention may be called to a parallelism between the effects of various foods upon the resistance of mice to acetonitrile and their efficiency in maintaining nitrogen equilibrium. Rubner[c] arranged a number of foods as follows with reference to the amount of protein in the food necessary to maintain nitrogen equilibrium and to yield 3,600 calories: Potatoes,[d] rice, maize, white bread, black bread, mother's milk, cow's milk, peas, eggs, meat.

Arranging the foods of the above experiments (Series 17 with the probable place of others in parentheses) in the order of their efficiency in protecting mice against acetonitrile, the following is obtained: Potatoes, (maize), (rice), (rye), bread (S), ham (lean), milk, eggs.

These diets stimulated growth in almost the reverse order.

It may be that this parallelism is only accidental, but it is suggestive of a deeper significance.

Sulphur, free and in certain forms of combination, increases the resistance of mice (and other animals) to acetonitrile; the question whether some of the results obtained in these experiments are due to this element in special forms of combination must be left to further studies. That sulphur can not be the determining factor in many cases, however, is shown by the fact that the same diet often affects mice on the one hand and rats and guinea pigs on the other in opposite ways, whereas sulphur has the same effect in all three cases.

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[a] In this connection brief mention may be made of a series of experiments performed several years ago to determine whether dextrose and other carbohydrates exert a protective action against acetone, the problem being suggested by the well-known clinical observations that in many cases of severe diabetes the acetone excretion is diminished and the symptoms improved by the administration of small amounts of carbohydrates. The experiments were performed upon rabbits and guinea pigs. The results were somewhat irregular, but they showed clearly that whereas rabbits died in from 3 to 8 days from daily doses of 3.8 to 4.1 gm. acetone per kilo weight, they lived for from 6 to 22 days when large doses of dextrose, cane, or milk sugar were administered with the acetone. The results were sufficiently striking to lead me to suggest that the administration of dextrose before chloroform anaesthesia might diminish the acetone excretion which usually follows such anaesthesia; the observations performed on this suggestion gave negative results. Beddard (Lancet, 1908, I, p. 782) has recently made a similar suggestion (from an entirely different standpoint, however) and at least one case has been reported (Weir, Lancet, 1909, II, p. 710) in which the administration of dextrose to a patient suffering from delayed chloroform poisoning seemed to have had a remarkably favorable effect. I also found rabbits to withstand slightly larger doses of ethyl alcohol and for longer periods when dextrose was administered simultaneously.

[b] Cf., for example, Cathcart, Jour. of Physiol., 1909, 39, p. 311; Shaffer and Coleman, Arch. of Internal Med., 1909, 4, p. 543.

[c] Rubner, Volksernährungsfragen, 1908, p. 19

[d] The potatoes contained 42 gm. protein, the rice 69 gms., the bread 85 gms., etc.

The methods described in this paper will probably prove useful in investigating some of the physiological problems of sulphur metabolism and in throwing light upon the forms of combination in which this element occurs in foods and the effect upon them of cooking, etc.

Relation of diet to the thyroid.—In addition to the various possible explanations enumerated above of the effects of different diets upon the resistance of animals to acetonitrile there remains to be considered the possibility that some of the diets influence the resistance by exerting an influence primarily upon the thyroid, which in turn influences other organs. I have reported elsewhere experiments which show that the changes in the resistance of animals to acetonitrile caused by the administration of iodine compounds is due, at least largely, to the effect of the iodine upon the thyroid, and it is generally accepted in clinical medicine that some of the effects frequently called "iodism" should more properly be called "thyroidism."[a] The recognition of this fact has led to experiments in exophthalmic goiter with food free from or very poor in iodine.

The question at once arose if the marked resistance to acetonitrile caused by certain diets might not be attributed to the effects of the iodine, which, as is well known, is contained to some extent in most articles of food.[b] This question has not been thoroughly examined, but there are several reasons for supposing that this factor is comparatively unimportant. Thus the effect produced by certain diets was often far greater than the maximum effect obtainable with very large amounts of iodine (cf., for example, the experiments in Series 81 below). There was, moreover, no parallelism between the degree of change in resistance and the amount of iodine reported to be present in the food. Thus, as has been shown above, both the yolk of egg and milk cause a very marked diminution in the resistance of mice to acetonitrile; yet the former is comparatively rich in iodine, containing, according to some analyses, nearly thirty times as much iodine as the latter. Oats and oatmeal, on the contrary, ordinarily contain an unusually small amount of iodine, but they cause a high degree of resistance.

These considerations directed my attention to another possibility, viz, that perhaps some of the diets contained substances especially adapted to building up the iodine free[a] part of the thyroid or stimulating in some way its growth; it is known from earlier experiments that such thyroid has a certain degree of physiological activity.

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[a] Cf. Heubner, Therap. Monatshefte, October, 1909.

[b] Bourcet, De l'iode dans l'organisme, Thèse, Paris, 1900; Monéry, Recherches nouvelles sur le fonction iodée de la glande thyroide, Thèse, Lyon, 1903; Dochez, Johns Hopkins Hospital Bull., 1908, 19, p. 235.

This was also suggested by the fact that I had found great differences between different groups of mice in the manner in which they reacted to the administration of iodine compounds; some groups of mice showed a greatly increased resistance to acetonitrile after the administration of potassium iodide or certain other iodine compounds, whereas other groups showed a much less or even no increased resistance after equal or larger doses of the same compound. There was, moreover, little relation between the amount of iodine fed and the degree of increased resistance attained. These facts suggested that the thyroid often varies in its ability to take up iodine or to make an active compound of it and that this is governed to some extent, at least, by the diet.

An examination of the literature showed that there is considerable evidence that the thyroids of both man and the lower animals vary much in different individuals in their ability to take up iodine. As is well known the administration of iodine compounds usually leads to an increase in the amount of iodine in the thyroid, but several writers have called attention to exceptions to this rule. Thus Neumeister[b] found no iodine in the thyroids of rabbits to which large doses of potassium iodide had been given daily for 14 days. Oswald[c] Monéry,[d] and Jolin[e] report cases in man in which large doses of potassium iodide administered shortly before death had not led to an increased amount of iodine in the thyroid; in some of these cases, however, the thyroids were probably very abnormal. Doctor Seidell and I also found that dogs varied much as to the amount of iodine their thyroids took up after the administration of iodoform and potassium iodide. Marine and Lenhart[f] have also found the thyroid to vary in its ability to take up iodine.[g]

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[a] By "iodine free thyroid" is meant here, as in an earlier publication (Bulletin 47 of the Hygienic Laboratory), thyroid which does not give even a qualitative test for iodine when 1 gm. of the sample is examined by the Baumann method. It is of course possible, or even probable, that such thyroid contains minute amounts of iodine, and it is possible that these are responsible for the physiological activity of such thyroid. The important point, however, is that such "iodine-free" or iodine-poor thyroid has but a low degree of physiological activity and that this is usually very greatly increased when iodine is administered to an animal.

[b] Neumeister, Lehrb. d. physiolog. Chemie, 2nd ed., 1897, p. 522.

[c] Oswald, Zeitschr. f. physiol. Chemie, 1897, 23, p. 298.

[d] Monéry, Recherches nouvelles sur la fonction iodée de la glande thyroide, Thèse, Lyon, 1903, p. 105.

[e] Jolin, Festschrift O. Hammarsten, 1906, p. 46.

[f] Marine and Lenhart, Archives of Internal Medicine, 1909, 4, p. 253.

[g] The contradictory results of the experiments upon the effect of iodine upon metabolism are probably due to a considerable extent to the thyroid gland reacting differently in different individuals.

These differences may not, however, depend entirely upon the amount of iodine—poor thyreoglobulin although in certain cases of goitre this is apparently the chief factor. Some agency (a ferment, e. g.) may be lacking which is necessary for the conversion of the iodine into a form which the thyroid can utilize.

In view of these facts I formulated the hypothesis that certain diets have constituents useful in building up material in the thyroid, which is able to combine with iodine, or which increase the iodine "receptors" of the thyroid. If this hypothesis is correct then it is to be expected that the administration of iodine to such animals would ordinarily lead to a still greater resistance to acetonitrile. And, conversely, it seemed probable that the diminished resistance to acetonitrile caused by certain diets (eggs and milk, for example) may be due to these diets causing excessive demands upon the thyroid by which certain elements of the latter may be largely exhausted. Thus it may be assumed, as a working hypothesis, that certain diets lead to an extensive elimination of iodine of the thyroid and a resultant diminution of the resistance to acetonitrile (in mice); if this is the case such thyroid should subsequently be able to take up iodine in large amounts with a rapid return to the normal resistance.

The following experiments give considerable support to both of these hypotheses:

Experimental.—That the effect upon the resistance of mice to acetonitrile of the administration of potassium iodide and some other iodine compounds differs markedly in different groups of mice according to the diet which they have received is shown by the following experiments.

Series 81. (Feb., 1908.) (x, 104.)

Groups of 15 young adult mice were placed upon the following diets for about 8 weeks. The only liquid given was water or water containing 0.2 per cent potassium iodide.

[Dose in mgm. per gm. mouse.]

Diet.	Weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
	Grams.		
a. Bread (S) and water	19.7	0.65	0.70
b. Bread and 0.2 per cent potassium iodide	18.3	.72	.77
c. Oatmeal and water	20.15	1.60	1.70
d. Oatmeal and 0.2 per cent potassium iodide	18.06	2.20	2.60

In these experiments not only was the resistance of mice fed upon a diet of oatmeal much greater than that of mice fed upon bread but the administration of potassium iodide with the latter diet led to but a slight increase in the resistance, whereas the effect in the case of the oatmeal-fed mice was marked.

Similar results were obtained in the following series.

Series 82. (Apr., 1910.) (xii, 42.)

Groups of young mice (10 in each group) were placed upon the following diets for about 5 weeks.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Bread (S)	Increased 5.51 per cent (from 12.7 to 13.4 gms).	0.80	0.90
b. Bread (C)	No change (12.7 gms.)	2.00	2.50
c. Egg (entire)	Increased 44.88 per cent (from 12.7 to 18.4 gms.).	.23	.26
d. Egg (entire)+0.1 per cent potassium iodide.	Increased 44.88 per cent (from 12.7 to 18.4 gms.).	.23	.26
e. Milk, raw	No change (12.65 gms.)	.19	.20
f. Milk, raw, and 0.1 per cent potassium iodide.	Increased 13.49 per cent (from 12.6 to 14.3 gms.).	.14	.16
g. Oatmeal	Increased 17.32 per cent (from 12.7 to 14.9 gms.).	.30	.35
h. Oatmeal and 0.1 per cent potassium iodide.	Increased 23.62 per cent (from 12.7 to 15.7 gms.).	1.00	1.1

Thus potassium iodide administered with egg or milk did not increase in the least the resistance of the mice to acetonitrile; in fact it seemed to diminish it in the case of the milk-fed mice. Oatmeal alone increased the resistance slightly; the slight effect was probably due to the short duration of the feeding. The administration of potassium iodide with the oatmeal increased the resistance very decidedly; the result was in sharp contrast with that obtained by feeding the iodide with egg or milk.[a]

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[a] The survivors of groups *a*, *c*, *d*, *e*, *f*, *g*, *h* were placed upon raw milk for about 10 days when their resistance to the nitrile was again determined. The earlier fatal doses are placed in parentheses for comparison.

[Dose in mgm. per gm. mouse.]

Previous diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Bread (S)	Increased 7.69 per cent (from 13 to 14 gms.)	0.10 (0.80)	0.18 (0.90)
c. Egg	Decreased 11.13 per cent (from 15 to 13.33 gms.)	(.23)	.13 (.26)
d. Egg and potassium iodide	No change (15.75 gms.)	.20 (.23)	.16 (.26)
e. Milk	No change (12 gms.)	(.19)	.07 (.20)
f. Milk and potassium iodide	No change (12.4 gms.)	(.14)	.10 (.16)
g. Oatmeal	No change (13.66 gms.)	(.30)	.15 (.35)
h. Oatmeal and potassium iodide.	Decreased 7.5 per cent (from 13.33 to 12.33 gms.).	.80 (1.00)	.90 (1.10)

The change to a milk diet caused a diminished resistance; this was least marked in the case of the mice which had received oatmeal and potassium iodide, thus affording another instance of the persistency of the increased resistance caused by this combination.

Age or long-continued feeding seems to have an effect upon the result of the administration of potassium iodide, as is shown by the following experiments:

Series 83. (Mar., 1908.) (x, 108.)

Groups of adult mice were fed as below for about 13 weeks:

[Dose in mgm. per gm. mouse.]

Diet.	Weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
	Grams.		
a. Bread and water	19.56	....	0.35
b. Bread and 0.2 per cent potassium iodide	22.87	....	.40
c. Oatmeal and water	22.27	2.10	2.30
d. Oatmeal and 0.2 per cent potassium iodide	21.95	2.00	2.20

The failure of the potassium iodide to increase the resistance of group *b* is probably to be explained on the absence of material in the thyroid capable of combining with the iodine. It is not so easy to offer an explanation of the failure of the potassium iodide to increase the resistance of group *d;* possibly the long-continued diet of oatmeal was of itself sufficient to increase the resistance to the maximum possible in these mice, and it is of interest to note that the fatal dose in this series was about the same as that in group *d*, series 81, where the potassium iodide had undoubtedly had an effect.

That the potassium iodide administered to group *d* of series 83 had an effect, however, is shown by the fact that when groups *c* and *d* were placed upon a diet of bread and water the members of this group retained their increased resistance longer than did those of group *c*. Thus the fatal dose for these groups after an exclusive diet of bread and water for 20 days was as follows:

[Dose in mgm. per gm. mouse.]

	Weight (average).	Recovered.	Died.
	Grams.		
c. (previously on oatmeal)	26.95	0.90	1.00
d. (previously on oatmeal and potassium iodide)	20.33	1.50	1.60

The diet of bread was continued for 3 weeks longer and the fatal dose again determined; the results were as follows:

[Dose in mgm. per gm. mouse.]

	Weight (average).	Recovered.	Died.
	Grams.		
c. (previously on oatmeal)	24.63		0.45
d. (previously on oatmeal and potassium iodide)	24.15	1.10	...

The continued high resistance of members of group *d* after they had been placed upon a diet of bread is probably to be explained by a storage of iodine during the period when they received potassium iodide.

There was, apparently, no storage of iodine by the mice which had originally received the diet of bread and potassium iodide, for when these were placed upon a diet of oatmeal, although their resistance was increased, this increase was not greater than it was in the case of those which had received no potassium iodide. The experiments were as follows: The mice of groups *a* and *b* (series 83) were placed upon a diet of oatmeal and water and their resistance to acetonitrile tested after 20 days. The results were as follows:

[Dose in mgm. per gm. mouse.]

	Weight (average).	Recovered.	Died.
	Grams.		
a (previously on bread and water)	20.11	0.50	1.00
b. (previously on bread and potassium iodide)	22.24	.50	1.00

After another interval of 20 days (the diet of oatmeal having been continued) a mouse of group *a* recovered from 1.2 mgm. per gm., showing a further increase in resistance.

In the following series also potassium iodide had no effect when administered with oatmeal. The mice were adults.

Series 84. (May, 1910.) (xii, 50.)

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average.)	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Milk, raw	Decreased 8.85 per cent (from 19.2 to 17.5 gms.).	0.10	0.11
b. Milk and 0.1 per cent potassium iodide	Decreased 4.14 per cent (from 19.3 to 18.5 gms.).	.15	.16
c. Oatmeal	No change (19.2 gms.)	.70	.70
d. Oatmeal and 0.1 per cent potassium iodide.	Decreased 6.63 per cent (from 19.3 to 18.13 gms.).	.70	.70

The difference in the resistance between the milk and the milk and potassium iodide fed mice was probably due to the potassium iodide checking the diminution of the resistance caused by the milk rather than to actual increase in the resistance.

Series 85. (Feb., 1908.) (x, 106.)

In this series young mice were kept upon diets of bread and oatmeal for about 8 weeks. One half of each group was then placed upon cakes and the other half upon cakes containing in each 4 gm., 0.05 gm. extract of bladderwrack (containing 0.086 per cent iodine). After 10 days acetonitrile was injected, with the following results:

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
A. Bread (original diet):			
(*a*) Cakes	Decreased 6.7 per cent (from 17.55 to 16.39 gms.)	0.55	0.65
(*b*) Bladderwrack	Decreased 11.6 per cent (from 18.86 to 16.47 gms.)	.60	.65
B. Oatmeal (original diet):			
(*a*) Cakes	Decreased 6.8 per cent (from 16.79 to 15.64 gms.)	1.80	2.20
(*b*) Bladderwrack	Decreased 2.4 per cent (from 17.62 to 17.19 gms.)	2.70	3.00

Thus the resistance of the bread-fed mice was not increased at all, or at least but very slightly, by the administration of bladderwrack; that of the oatmeal-fed mice was much increased (probably by at least 40 per cent).

The following series also shows the increased resistance to acetonitrile caused by the administration of an iodine compound to oatmeal-fed mice:

Series 86. (Feb., 1908.) (x, 105.)

The mice had been fed for about 8 weeks on a diet of oatmeal and milk; their resistance to acetonitrile was as follows: Weight (average), 23.72 gms.; recovered, 2.3; died, 2.6.

Half of the mice were placed upon "plain cakes" and half upon cakes containing 0.05 gm. bladderwrack (with 0.086 per cent iodine) each; after 11 days they were again tested with acetonitrile with the following results:

[Dose in mgm. per gm. mouse.]

Diet.	Weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
	Grams.		
a. Cakes	20.18	1.80	2.00
b. Bladderwrack, 0.05+4	21.26	3.00	3.10

All of the above series were performed in February and March, that is, in months when the natural resistance of mice to acetonitrile is high. The following series was performed in June, when the resistance is low:

Series 87. (June, 1908.) (x, 198.)

The mice of this series consisted of adults, many of which had in the late winter or early spring been used for other purposes. On the 4th of April one-half (Group *A*) were placed upon an exclusive diet of bread and water, the other half (Group *B*) upon a diet of oats and water. After about 8 weeks (June 2) each group was divided into four subgroups and one subgroup placed upon each of the following diets: (*a*) cakes, (*b*) cakes + 0.05 gm. extract of bladderwrack, (*c*) cakes + 0.02 gm. iodoform, (*d*) cakes + 0.02 gm. potassium iodide. About a week later the resistance of members of each group was tested to acetonitrile; the results were as follows:

GROUP A (BREAD FED.)

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Cakes	Decreased 12.5 per cent (from 19.52 to 16.89 gms.)	0.11	0.12
b. Bladderwrack, 0.05+4	Decreased 11 per cent (from 20.67 to 18.40 gms.)	.32	.35
c. Iodoform, 0.02+4	Decreased 15.6 per cent (from 18.66 to 15.74 gms.)	.40	.42
d. Potassium iodide, 0.02+4	Decreased 9.8 per cent (from 19.07 to 17.20 gms.)	.52	.55

GROUP B (OATS FED.)

[Dose in mgm. per gm. mouse.]

a. Cakes	Deceased 0.8 per cent (from 18.42 to 18.27 gms.)	0.40	0.43
b. Bladderwrack, 0.05+4	Decreased 2.7 per cent (from 16.33 to 15.89 gms.)	.65	.68
c. Iodoform, 0.02+4	Decreased 6.4 per cent (from 16.75 to 15.68 gms.)	.77	.80
d. Potassium iodide, 0.02+4	Decreased 4.7 per cent (from 18.44 to 17.58 gms.)	.70	.72

The administration of iodine compounds led to an increased resistance in the case of both the bread-fed and the oats-fed mice, or perhaps (especially in the case of the latter) had prevented a diminution of the resistance. The relative increase in resistance was greater in the case of the bread-fed mice, but the degree of resistance reached was greater in the oats-fed animals; hence the results are in harmony with the hypothesis that a diet of oats leads to the formation of a larger amount of iodine-free thyreo-globulin.

It is difficult to suggest a satisfactory explanation of why the administration of iodine compounds to the bread-fed mice in this series (in June) led to an increased resistance whereas it was practically without effect in the earlier series (February and March).

The difference may have been due to differences in the age of the mice, or in their previous treatment, but it seems more probable that it is connected in some way with the seasonable variations in resistance to which attention has already been called. An observation of F. C. Koch[a] is suggestive in this connection; Koch found sheep thyroids to invariably assay higher in iodine (as much as three times) during the winter months than during June and July.[b] The most plausible explanation of this interesting observation of Koch would, at first thought, seem to be that this difference was the result of different methods of feeding during the winter and early summer (the administration of iodine-containing salt, for example) but this explanation should not be accepted without evidence. Certainly my results showing that mice are least resistant to acetonitrile during the summer months and the results of the experiments of series 87, showing that in June the administration of iodine compounds to bread-fed mice increases their resistance (due, I believe, to the ability of the thyroid to take up iodine at this season), suggest that at this season the iodine of the thyroid is abnormally low or the diet of bread may have led to an exhaustion or reduction of the iodine already present in the gland. Investigations may show that the differences in the iodine content of sheep thyroids observed by Koch may not be due to differences in diet but to diminution of the iodine normally stored in this gland and that this diminution may have resulted from the demands made upon the thyroid by other organs or processes of metabolism during the spring months.[c]

Series 88. (Jan., 1910.) (xii, 2.)

Two groups of young, almost grown mice, 25 in each, were fed, one on a diet of bread and oats and the other upon mush made from corn meal, from November 6, 1909, to December 20, when their resistance to acetonitrile was determined.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
A. Bread (C) and oats........	Increased 26.2 per cent (from 13.80 to 17.42 gms.).....	1.20	1.30
B. Corn meal mush..........	Decreased 3.8 per cent (from 13.80 to 13.28 gms.)......	.80	.90

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[a] Koch, Proc. Amer. Pharm. Assoc., 1907, 55, p. 371.

[b] Monéry (Recherches nouvelles sur la fonction iodeé de la glande thyroide. Thèse. Lyon. 1903) and Mossé found the thyroids of sheep in parts of France to contain more iodine in the winter than in the summer; although they speak of this variation being due to the season they apparently consider the different kinds of food received at different periods as having at least an important part.

[c] It would be interesting to determine, for example, if there is a relation between the iodine of the thyroid and the composition of milk; the latter contains more fat and protein in the winter than in the summer months.

The remaining mice in each group were divided into three subgroups and one of the latter placed upon one of the following diets: (*a*) Cakes, (*b*) cakes containing 0.05 gm. extract of bladderwrack (containing about 0.03 per cent iodine), and (*c*) cakes containing 0.01 gm. potassium iodide. After about 10 days their resistance to acetonitrile was again determined.

GROUP A (BREAD AND OATS-FED).

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Cakes	Decreased 14.3 per cent (from 19.6 to 16.8 gms.)	0.35	0.45
b. Bladderwrack, 0.05+4	Decreased 11.7 per cent (from 17.5 to 15.5 gms.)	3.60	....
c. Potassium iodide, 0.01+4	Decreased 14 per cent (from 19.3 to 16.6 gms.)	1.60	1.70

The change from a diet of bread and oats to one of cakes led to a diminished resistance. Bladderwrack had caused a markedly increased resistance; potassium iodide had also caused an increased resistance, but this was less than that caused by the bladderwrack,[a] although the potassium iodide contained about five hundred times as much iodine as the latter.

GROUP B (CORN MEAL FED).

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Cakes	Decreased 7 per cent (from 11.97 to 11.13 gms.)	0.67	0.70
b. Bladderwrack, 0.05+4	Decreased 9.9 per cent (from 14.58 to 13.13 gms.)	......	.65
c. Potassium iodide, 0.01+4	Decreased 15.1 per cent (from 14.73 to 12.5 gms.)	1.05	1.10

The change from corn meal mush to cakes led to a slight diminution in resistance. The administration of bladderwrack caused no increase in the resistance, whereas potassium iodide had caused an increased resistance.

Comparing the effects of the iodine compounds in Groups *A* and *B*, it is evident that they were more effective in increasing the resistance of the mice which had received a diet containing oats than of those which had received a diet of corn meal mush.

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[a] The iodine in bladderwrack is usually much more effective in causing an increased resistance to acetonitrile than that in other compounds with which I have experimented; the iodine compound in bladderwrack seems to have a special affinity for the thyroid. This subject will be discussed in more detail in a publication, by Doctor Seidell and myself, in the Journal of Pharmacology and Experimental Therapeutics, 1910, vol. 2.

Series 89. (Jan., 1910.) (xii, 31.)

Two groups of young, nearly grown, mice were fed, one (Group *A*) upon an exclusive diet of boiled eggs, the other (Group *B*) upon corn meal mush, from November 16 to December 22, 1909, when their resistance to acetonitrile was determined. The results were as follows:

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
A. Egg	Increased 10.7 per cent (from 16.33 to 18.08 gms.)	0.85	0.95
B. Corn meal mush	Decreased 16.9 per cent (from 16.33 to 13.57 gms.)	.55	.80

The remaining mice of each group were divided into two subgroups, one of which was placed upon a diet of simple cakes and the other upon cakes containing 0.01 gm. potassium iodide. After 10 days their resistance to acetonitrile was again determined, with the following results:

GROUP A (EGG-FED).

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Cakes	Decreased 9.6 per cent (from 20.17 to 18.24 gms.)	2.0	2.5
b. Potassium iodide, 0.01+4	Decreased 10.4 per cent (from 17.7 to 15.86 gms.)	.8	1.1

It is evident that the potassium iodide had had no effect upon the resistance of the mice. I am unable to explain the unexpected and unusual increase in the resistance of the mice which had received the cakes. It is improbable that it was due to an experimental error, for the same solution of acetonitrile was used in both series and the experiments were performed upon the same days; 4 of the cakes-fed mice recovered and none died from doses of the nitrile ranging from 1 mgm. to 2 mgm. per gm., whereas 5 of the potassium iodide-fed mice died, and none recovered from doses ranging from 1.1 mgm. to 2 mgm. per gm. There is a possibility, however, that cakes containing bladderwrack instead of simple cakes had been fed.

GROUP B (CORN MEAL MUSH).

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Cakes	Decreased 8.7 per cent (from 15.48 to 14.13 gms.).....	0.7	0.75
b. Potassium iodide, 0.01+4..	Decreased 10.4 per cent (from 13.43 to 12.03 gms.)....	1.2	1.50

Thus potassium iodide had caused a somewhat increased resistance. A few weeks later the following series was performed:

Series 90. (Mar., 1910.) (xii, 36.)

Young mice were fed upon diets of bread and eggs; the bread was the brand designated "C," which always caused a high degree of resistance. After about 5 weeks acetonitrile was injected with the following results:

[Dose in mgm. per mg. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Bread (C)..	None..	4.30	4.40
b. Eggs.	Increased 34.9 per cent (from 12.6 to 17 gms.)........	.35	.45

The egg diet had led to a low degree of resistance. It is interesting to compare the results obtained in this series with those obtained in series 89, performed but a few weeks earlier; in the latter the egg diet did not cause a low resistance. The explanation of this difference is probably to be found in the differences in the effects upon the growth in the two cases; in series 89 the mice had increased by but 10.7 per cent in weight; in series 90 by 34.9 per cent.

The survivors of group *a* were divided into two subgroups and placed upon diets of cakes, and cakes containing potassium iodide; those of group *b* were divided into three groups and placed upon cakes, and cakes containing extract of bladder-wrack (with about 0.03 per cent iodine) or potassium iodide. After about 11 days their resistance to acetonitrile was again tested with the following results:

GROUP A (BREAD FED).

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Cakes	Decreased 13.9 per cent (from 12 to 10.33 gms.).......	2.2	3.0
b. Potassium iodide, 0.01+4..	Decreased 7.4 per cent (from 11.46 to 10.61 gms.)	4.0	4.5

The mice on a diet of cakes had lost part of their high degree of resistance; those receiving the potassium iodide had retained it.

GROUP B (EGG FED).

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of acetonitrile.	
		Recovered.	Died.
a. Cakes	Decreased 12 per cent (from 17.67 to 15.54 gms.)	0.12	0.3
b. Bladder-wrack, 0.05+4	Decreased 12.5 per cent (from 16.59 to 14.5 gms.)	.40	.8
c. Potassium iodide, 0.01+4	Decreased 15.5 per cent (from 16.97 to 14.34 gms.)	1.00	1.1

The resistance of the mice receiving the diet of cakes was still further lowered; that of those receiving the bladder-wrack was not much changed, whereas those receiving the potassium iodide was increased.

SUMMARY OF THE ABOVE EXPERIMENTS.

These experiments lend considerable support to the hypothesis suggested above, viz, that in some cases the effect of diet upon the resistance of mice to acetonitrile is exerted, at least in part but probably only in part, through the thyroid gland. The experiments with oats and oatmeal and eggs are of especial interest. In the earlier parts of this paper many experiments were quoted showing that a diet of oatmeal or of oats usually leads to a marked resistance of mice to acetonitrile; the experiments quoted in this section, which show that the administration of certain iodine compounds with or subsequently to such a diet still further increases this resistance, and the experiments previously reported showing that as far as the resistance toward acetonitrile is concerned iodine exerts its action through the thyroid gland, all point to the conclusion that the resistance caused by an oat diet is in part an effect exerted upon the thyroid. This effect is obtained much more markedly and constantly with young, growing mice.

From these experiments and considerations it seems very probable that it is possible to influence, in a specific manner, by diet, one of the most important hormones in the body; this is a comparatively new principle in dietetics and one which may prove of much importance.[a]

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[a] After the completion of most of the above experiments my attention was called to an article by Watson (Lancet, 1907, I, p. 985) in which the author reports that the thyroid of young rats which have been fed on oatmeal are enlarged; he states that the "results appear to indicate that an excessive oatmeal diet has a markedly stimulating effect on the thyroid gland of young animals."

Oats have long been held to have a "stimulating" action upon animals. Pugliese and Brighenti (Jour. de physiol. et de la pathol. gén., 1909, 11, p. 1017) have recently found that the products of the autolysis of oats and of their peptic digestion increases

There are also a number of facts which support the hypothesis that the effect of the egg diet upon the resistance of mice to acetonitrile is due in part to an effect upon the thyroid. Thus the egg diet has an effect just the reverse of that of the administration of thyroid; moreover, the marked increase in the resistance of mice which have had such a diet, which sometimes occurs when potassium iodide is subsequently administered, suggests that the egg diet had led to a condition of exhaustion of the gland.

It is not necessary to assume that this change in the thyroid is the direct effect of the egg diet; it would be more logical to suppose that it is an indirect effect, probably an effect of the rapid growth caused by the egg diet. It is known that a normally functioning thyroid is necessary for the growth of an animal, and it is readily conceivable that a diet which causes an abnormally rapid growth might lead to a temporary exhaustion of the gland. It is interesting to recall in this connection that with the egg diet the lowest resistance was found in those cases in which there had been the most rapid growth; with a less rapid growth there was a higher resistance. It is possible, however, to select a combination of foods which will cause rapid growth and still maintain a rather high degree of resistance to acetonitrile (see below).

These considerations are almost purely theoretical[a]; they are, for the most part, however, open to experimental proof or refutation. But whether the suggested explanations prove true or false the experiments show that it is possible to classify a number of ordinary foods as to whether they affect the organism of the mouse in the same way or in the opposite way as does thyroid. Oats, oatmeal, liver, kidney, dextrose, and certain kinds of wheat bread belong to the former class; milk and eggs to the latter class.

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the strength of the contractions of the heart and of striated muscle and delay the fatigue of the latter.

I have made, with Doctor Seidell, a few preliminary experiments in an endeavor to determine what constituents of oats are responsible for the effect upon the thyroid. From the experiments of series 17 it will be seen that the ash of oats added to bread did not alter the latter's effect upon the resistance of mice to acetonitrile. In series 26 the aqueous extract of oats added to corn meal seemed to lower slightly the resistance of mice as compared with corn meal and water.

From series 18 it would seem as if the constituents causing the increased resistance were removed by 96 per cent alcohol.

[a] Note during proof reading. The correctness of these conclusions has been proved by feeding the thyroid of mice (kept on certain diets) to other mice and testing the resistance of the latter to acetonitrile. The mice which were most resistant to this poison had thyroids which, when fed to other mice, afforded the greatest degree of protection. These experiments give the direct proof that in some cases the resistance of mice to acetonitrile is due to the activity of the thyroid and also that the acetonitrile test is a measure (at least to a certain extent) of the normal physiological activity of the thyroid in the body. These results will be published in detail shortly.

The question whether these foods affect the human organism as they do that of the mouse must be left for future study; the probabilities are that they do. Careful observations upon man in conditions of hypo- and hyperthyroidism would throw much light upon this subject; such observations, however, do not seem to have been made. Berkeley's remarks [a] upon the great value of eggs and milk in the treatment of exophthalmic goitre and the advisability of eliminating sweets in this disease are very suggestive; it would be interesting to know if certain other foods which experimentally have an effect similar to thyroid (oatmeal, liver, kidney) are contraindicated in this disease. The experiments are of interest in connection with the iodine-free-food treatment which has been advocated for this disease. It is generally recognized that iodine usually aggravates the symptoms in exophthalmic goitre. These experiments suggest that diet is capable of causing much greater changes in the activity of the thyroid than iodine or any other drug and that more careful study should be given it.

The experiments also show how some effects of one food may be retained and other effects more or less eliminated by combining it with other foods. Thus the effect of egg yolk in causing rapid growth is retained when oatmeal or liver is added, but the latter reduces to a considerable extent its property of increasing the susceptibility to acetonitrile. Egg white added to egg yolk had no effect upon either the rate of growth or resistance to acetonitrile.

The above results are suggestive in connection with the oatmeal treatment of diabetes mellitus. It is very probable that hormones other than the thyroid can be influenced in a definite manner by certain diets; the hypothesis that the favorable results obtained in certain cases of diabetes by an oatmeal diet are due to the latter having a special relation to some of the hormones involved in the metabolism of sugar seems much more probable than some of the other hypotheses which attribute them to differences in the carbohydrates.

The experiments on the effect of diet upon the result of the administration of iodine compounds are of interest in another connection: One of the marked tendencies in pharmacology at present (due to a considerable extent to the influence of Ehrlich) is the endeavor to find drugs which have special affinities for certain organs or parts of organs, drugs which are neurotropic, lipotropic, thyreotropic, etc. As will be shown in a later publication there are certain iodine compounds which seem to be very distinctly thyreotropic. The observations reported above (and others of a similar character to be reported later) suggest that it is possible to modify, by diet, organs so that they may more readily take up certain drugs and it seems that in certain cases more pronounced results may be obtained in this way than by changing the chemical constitution of the drug.

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[a] Berkeley, Johns Hopkins Hospital Bulletin, 1908, 19, p. 259.

Newman (Lancet, 1909, II, p. 1584) reports a case of exophthalmic goitre in which sour milk seemed to be beneficial; the souring would largely eliminate the sugar which according to Berkeley seems to be contraindicated and which according to my experiments has an effect somewhat similar to small doses of thyroid. The beneficial effects noted by Edmunds (Lancet, 1908, I, p. 227) and by Goebel (Münch. med. Woch., 1902, p. 835), from the administration of comparatively large amounts of the milk of thyroidectomized goats in cases of exophthalmic goitre may have been due in part to the milk *per se* as well as to the presence of an antibody or the absence of iodine.

b. EXPERIMENTS WITH GUINEA PIGS.

Only a small number of experiments were performed upon guinea pigs with the special object of determining if a relation exists between the diet and the resistance of these animals to acetonitrile; the results of these experiments were, however, positive. The experiments brought out a number of other points of some interest; some of these will be referred to briefly.

Effect of season.—The fatal dose of acetonitrile for guinea pigs has, during the last 3 or 4 years, been determined several times in nearly every month; the average of these determinations, expressed in mgms. per gm. body weight, is as follows:

January	0.2	June	0.42
February	.25	July	.45
March	.35	September	.40
April	.35	November	.20
May	.35	December	.26

Thus the animals were most resistant in the spring and summer months, i. e., at a time when mice were the least resistant. Since removal of the thyroid tends to increase the resistance of guinea pigs to acetonitrile and the administration of iodine compounds (by which the activity of the thyroid is increased) diminishes the resistance it is probable that an important factor in these seasonable variations are variations in the activity of the thyroid gland; this conclusion is in harmony with the results of experiments on mice and the variations in the iodine content of sheep thyroids already discussed.

We are not in possession of sufficient data to attempt to explain these variations but certain factors may be mentioned. First among these is the diet. All of the guiena pigs were bred in the laboratory and their parentage and diet were known. The diet consisted of dry oats, bran, hay, and "green food;" the latter consisted in the winter and early spring (from November to May) of carrots and cabbage; in the summer it consisted of green rye, alfalfa, green maize, or carrots and cabbage. The animals had access to rock salt throughout the year. Although the green food of the winter months may have contained a little more iodine (which lowers the resistance of guinea pigs to acetonitrile) than did that of the summer it seems improbable that this or the other slight differences in diet was the cause of the differences in susceptibility. It seems more probable that the latter is associated in some way with seasonable variations of metabolism of unknown origin.

Physiological oxidation processes are, as is well known, increased by cold and the question may arise if the increased susceptibility during the winter months may not be due to accelerated metabolism which would lead to a greater decomposition of the acetonitrile molecule. But, as Rubner has shown, the coat of hair on animals like the clothing of man, largely or totally abolishes the effect of moderate cold upon metabolosm. Moreover the guinea pigs were kept in a uniformly heated room throughout the winter and were not exposed to greater differences in temperature than were the mice used in the preceding experiments, the resistance of which was increased during the winter.

A factor which should receive more careful consideration is the possible relation between susceptibility to acetonitrile and rate of growth. Südmersen and Glenny[a] found the average age of 250 gm. guinea pigs in the 6 months of winter (October to March) to be 44.6 days as compared with 32.8 days in summer. They also found that a larger dose of diphtheria toxin was necessary to kill a guinea pig of the same weight in summer than in winter and that for guinea pigs of the same weight the fatal dose increased with the age.

Effect of feeding thyroid.—The feeding of thyroid lowers the resistance of guinea pigs to acetonitrile, as is shown, for example, by the following experiments:

Series 91. (Sept., 1908.) (xi, 90.)

The thyroid was fed in the form of commercial tablets, three different products being used. The thyroid was fed for 13 days.[b]

A. CONTROLS.

[Dose in mgm. per gm. guinea pig.]

No.	Change in weight.	Fatal dose of acetonitrile.	
		Recovered.	Died.
1	Increased 34.9 per cent (from 235 to 317 gms.)	0.38	
2	Increased 44.1 per cent (from 215 to 310 gms.)	.40	
3	Increased 40.4 per cent (from 205 to 288 gms.)		0.40
4	Increased 32.6 per cent (from 230 to 305 gms.)		.43
5	Increased 34.6 per cent (from 225 to 303 gms.)		.50

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[a] Südmersen and Glenny, Jour. of Hygiene, 1909, 9, p. 399.

[b] The effect of sulphur upon the resistance of guinea-pigs to acetonitrile was tested at the same time, the results will be given here in order to avoid repetition.

B. 0.128 GM. THYROID CONTAINING 0.113 PER CENT IODINE (=0.1446 MGM. IODINE) FED DAILY.

[Dose in mgm. per gm. guinea pig.]

No.	Changes in weight.	Fatal dose of acetonitrile.	
		Recovered.	Died.
1	None (210 gms.)	0.20	
2	Increased 13.6 per cent (from 220 to 250 gms.)	.30	
3	Increased 7.7 per cent (from 220 to 237 gms.)		0.35
4	Increased 5.6 per cent (from 230 to 243 gms.)		.40

C. [a]0.064 GM. THYROID WITH 0.38 PER CENT IODINE (=0.2432 MGM. IODINE) FED DAILY.

[Dose in mgm. per gm. guinea pig.]

1	Increased 13 per cent (from 230 to 260 gms.)	0.3	
2	Increased 2.3 per cent (from 215 to 220 gms.)		0.35
3	None (235 gms.)		.40

D. 0.128 GM. THYROID WITH 0.065 PER CENT IODINE (=0.0832 MGM. IODINE) FED DAILY.

[Dose in mgm. per gm. guinea pig.]

1	Increased 13 per cent (from 230 to 260 gms.)	0.25	
2	Increased 11.8 per cent (from 235 to 263 gms.)		0.30
3	Increased 14.4 per cent (from 215 to 246 gms.)		.32
4	Decreased 7.8 per cent (from 205 to 189 gms.)		.40

E. 0.2 GM. SULPHUR IN THE FORM OF PILLS WAS FED DAILY.

[Dose in mgm. per gm. guinea pig.]

1	Increased 3.2 per cent (from 245 to 253 gms.)	0.45	
2	Increased 12 per cent (from 240 to 269 gms.)		0.55

[a] Another guinea pig of this series died on the sixth day after the administration of thyroid was begun.

It is evident that the thyroid had in all these cases very distinctly diminished the resistance of the guinea pigs to acetonitrile. The thyroid also caused a retardation of growth. The latter effect was roughly proportional to the percentage of iodine in the thyroid fed; this is another indication that the activity of the thyroid is parallel to the iodine content. I have shown in earlier papers that the increased resistance of mice to acetonitrile caused by the feeding of thyroid is very closely parallel to the iodine content of the latter; this did not hold in these experiments on guinea pigs for the diminished resistance caused by the feeding of thyroid. It is quite probable that the increased resistance caused in mice is a primary effect of the thyroid, whereas the diminished resistance in the guinea pigs is an effect secondary to other effects.

Sulphur caused a distinct increase in the resistance to acetonitrile; the effect was not nearly as great as it was in similar experiments on mice (series 66, for example) and rats.

Oats.—It has been shown above that a diet of oats as compared with many other diets increases the resistance of mice to acetonitrile, and arguments were presented that this effect is probably due in part at least to a stimulating action on the thyroid. Since thyroid and potassium iodide and other iodine compounds administered to guinea pigs diminish their resistance to acetonitrile, it would be expected that oats would have a similar effect if it had a stimulating action upon the thyroid similar to that observed in mice.[a] This supposition was realized in the two series of experiments I have performed.

Series 92. (Mar., 1908.) (x, 121.)

Two groups of guinea pigs of approximately the same age and weight were fed from January 17 to March 7, as follows: Members of Group *A* received an unlimited amount of oats and in addition 5 gms. of carrots and cabbage daily for the first 25 days and thereafter the same amount every second day.[b] Group *B* received only cabbage and carrots (equal parts).

A. (OATS FED.)

[Dose in mgm. per gm. guinea pig.]

No.	Change in weight.	Fatal dose of acetonitrile.	
		Recovered.	Died.
1	Increased 45.8 per cent (from 230 to 340 gms.)	0.35	
2	Increased 5.7 per cent (from 225 to 238 gms.)		0.37
3	Increased 3.7 per cent (from 240 to 249 gms.)		.40
4	Increased 54.5 per cent (from 240 to 371 gms.)		[a].45

B. (GREEN FOOD.)

No.	Change in weight.	Recovered.	Died.
1	Increased 12.9 per cent (from 240 to 271 gms.)	0.40	
2	Increased 31 per cent (from 235 to 308 gms.)	.45	
3	Increased 6.9 per cent (from 245 to 262 gms.)	.47	
4	Increased 23.8 per cent (from 235 to 291 gms.)	.47	
5	Increased 17.7 per cent (from 225 to 265 gms.)		0.50
6	Increased 11.3 per cent (from 230 to 256 gms.)		.55

[a] Two others died from 0.45 mgm. per gm. weight.

Series 93. (July, 1907.) (x, 120.)

The guinea pigs were fed for about a month as in series 92, except that the group on oats received a few grams of green food but twice a week.

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[a] I do not believe that the reaction of guinea pigs to acetonitrile is by any means as closely related to thyroid activity as that of mice seems to be. Diet, etc., may and probably does affect the thyroids of both animals in the same way, but there is no means of accurately determining the effect in guinea pigs.

[b] As is well known, guinea pigs do not live long upon an exclusive diet of oats, but a very small amount of green food enables them to thrive on it.

A. (OATS.)

[Dose in mgm. per gm. guinea pig.]

No.	Change in weight.	Fatal dose of acetonitrile.	
		Recovered.	Died.
1	Decreased 4 per cent (from 250 to 240 gms.)	....	0.27
2	Increased 42.2 per cent (from 225 to 320 gms.)	....	.30
3	Increased 27.4 per cent (from 255 to 325 gms.)	....	.30
4	Increased 38.2 per cent (from 235 to 325 gms.)	....	.38

B. (GREEN FOOD.)

No.	Change in weight.	Recovered.	Died.
1	Increased 66.6 per cent (from 240 to 400 gms.)	0.36	....
2	Increased 72.9 per cent (from 240 to 415 gms.)	.42	....
3	Increased 78.7 per cent (from 235 to 420 gms.)	.47	....
4	Increased 64.6 per cent (from 235 to 383 gms.)	.53	....

These experiments show that the resistance of guinea pigs to acetonitrile is influenced by the diet. The results are also in harmony with the hypothesis that a diet of oats has a special effect upon the thyroid.

Attention has already been called to the fact that the administration of prostate to guinea pigs diminishes their resistance to acetonitrile; one of the series of experiments on this subject was as follows:

Series 94. (May, 1907.) (vii, 25.)

Group *B* received from 0.38 to 0.51 gms. dried prostate daily for 13 days. The changes in weight and the fatal dose of acetonitrile were as follows:

A. CONTROLS.

[Dose in mgm. per gm. guinea pig.]

No.	Change in weight.	Fatal dose of acetonitrile.	
		Recovered.	Died.
1	Increased 12.2 per cent (from 490 to 550 gms.)	0.28	....
2	Increased 9.1 per cent (from 240 to 262 gms.)	....	0.31
3	Increased 18 per cent (from 250 to 295 gms.)	....	.34

B. PROSTATE.

No.	Change in weight.	Recovered.	Died.
1	Increased 11.4 per cent (from 245 to 273 gms.)	....	0.19
2	Increased 13.7 per cent (from 290 to 330 gms.)	....	.23

Thus prostate has an effect upon the resistance of guinea pigs to acetonitrile similar to that of thyroid.

(c.) EXPERIMENTS ON DOGS.

Only one experiment was made upon dogs to determine if diet has an influence upon the excretion of sulphocyanate after the administration of acetonitrile.

Series 95. (Jan. 6, 1906.) (vii, 9–10 and vi, 150–151.)

Two bitches of approximately the same weight were fed for about 3 weeks on a diet consisting of 50 gms. lean beef and as many Spratt's dog biscuit as they would eat. Acetonitrile, 0.1 mgm. per gm. weight, was then injected into each. The sulphocyanate in the urine was determined, by Lang's method, for five days; in one dog (*A*) 12.6 per cent of the cyanogen of the nitrile appeared in the urine as sulphocyanate; in the other (*B*) 14.7 per cent. Lang recovered in similar experiments upon dogs (diet not stated) 14 and 18.4 per cent of the cyanogen.[a] Dog *A* was then placed upon a diet consisting exclusively of lean beef, and dog *B* upon a diet consisting of boiled rice, cane sugar, and lard in the following proportions: 400:300:50. After 17 days acetonitrile, 0.2 mgm. per gm. animal, was injected into each dog. The meat-fed dog showed in a short time fairly marked symptoms of poisoning; the carbohydrate-fat fed dog showed almost no symptoms until the following day. Sixteen and nine-tenths per cent of the cyanogen of the nitrile administered to dog *A* (meat fed) was recovered from the urine; only 9.5 per cent of that given to dog *B* was recovered.

Thus the meat-fed dog showed somewhat severer symptoms of intoxication and excreted more (1.7 times as much) of the cyanogen of the nitrile as sulphocyanate than did the carbohydrate-fat fed dog. This result may have been due either to the meat-fed dog having decomposed a larger proportion of the nitrile (owing to increased metabolism) or it may have been due to the meat having made possible the formation of a larger amount of sulphocyanate through its larger sulphur content;[b] experiments with hydrocyanic acid would probably have determined this.

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[a] In two series of experiments upon rats 11.8 per cent and 12.8 per cent of the cyanogen contained in the injected acetonitrile was recovered from the urine as sulphocyanate.

[b] The meat-fed dog excreted on this diet a daily average of 3.475 gms. total sulphur (expressed as barium sulphate), of which 2.606 gms. (or 75 per cent) was in the form of sulphates and 0.869 gm. as neutral sulphur. (On the earlier mixed diet 64.1 per cent of the sulphur was in the form of sulphates.) The carbohydrate-fat-fed dog excreted but 0.395 gm. (expressed as barium sulphate) total sulphur, of which 0.1373 gm. (34.8 per cent) was in the form of sulphate and 0.2577 gm. as neutral sulphur. The meat-fed dog thus had a much larger amount of sulphur available for the formation of sulphocyanate if the sulphur of meat is in a form available for this purpose.

2. EXPERIMENTS WITH PROPIONITRILE.

Experiments with mice.—Propionitrile (C_2H_5CN) is much more poisonous than is acetonitrile, and the resistance of mice to it is greatly influenced by the diet, as is shown by the following experiments:

Series 96. (June, 1908.) (x, 176.)

The mice belonged to the same groups as those of Series 17; they were fed as below for about 75 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of propionitrile.	
		Recovered.	Died.
a. Lean ham	Increased 26 per cent		0.004
b. Milk, raw	Increased 13 per cent	0.005	.007
c. Egg yolk	Increased 39 per cent	.008	.010
d. Milk, boiled 30 minutes	Increased 25.6 per cent	.008	.012
e. Bread and oat ash [a]	Increased 5.7 per cent	.010	.025
f. Potato, boiled	Decreased 7.9 per cent		.015
g. Bread and water	Increased 16.4 per cent	.017	.018
h. Oats and water	Increased 22.9 per cent		.024
i. Cheese	Increased 14.6 per cent	.032	.034
j. Liver (hog), boiled	Increased 10.5 per cent	.050	.054

[a] The mice received daily the ash from about 9 gms. oats intimately mixed with the bread.

In comparing the fatal doses of propionitrile with those of acetonitrile (series 17) it will be seen that the latter are from thirty to sixty times as great as the former. Diet had a pronounced effect upon the fatal dose of propionitrile; this effect was in several cases different from that caused by the same diet upon the toxicity of acetonitrile.

Series 97. (July, 1908.) (xi, 2.)

The mice of this series were of the same groups as those of series 18. The mice were fed as below for 5 weeks.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of propionitrile.	
		Recovered.	Died.
a. Egg yolk and liver, equal parts.	Increased 23.2 per cent	0.010	0.015
b. Egg, entire	Increased 22.5 per cent	.015	.016
c. Egg, yolk	Increased 24.2 per cent	.015	.016
d. Oats, extracted [a]	Decreased 16.0 per cent	.013	.016
e. Egg white and oatmeal, equal parts.	Increased 10.2 per cent	.016	.020
f. Brain (sheep, boiled) and oatmeal, equal parts.	Increased 14.7 per cent	.023	.025
g. Oatmeal	Increased 5.7 per cent	.027	.028
h. Liver (hog), boiled	Increased 1.3 per cent		.030
i. Kidney (sheep), boiled	Increased 20.4 per cent	.042	.044
j. Egg white and liver, equal parts	Increased 2.0 per cent	.045	.049

[a] Oats were ground and the meal, after sifting, was extracted with 90 per cent alcohol.

The influence of diet upon the resistance of the mice to propionitrile is apparent; the effect was in several instances different from that observed with acetonitrile.

EFFECT OF IODINE COMPOUNDS.

Series 98. (June, 1908.) (x, 198.)

One group of mice (*A*) was kept for 8 weeks upon a diet of bread and water; another group (*B*) upon oats and water. Each group was then divided into subgroups which were fed as below for about a week when their resistance to propionitrile was tested.[a]

GROUP A (BREAD-FED).

[Dose in mgm. per gm. mouse.]

Diet.	Fatal dose of propionitrile.	
	Recovered.	Died.
a. Cakes	0.018	0.020
b. Bladderwrack, 0.05+4	.025	.026
c. Iodoform, 0.02+4	....	.026
d. Potassium iodide, 0.02+4	.020	.022

Thus bladderwrack caused a distinct increase in resistance; the results with iodoform and potassium iodide were doubtful.

GROUP B (OATS-FED.)

[Dose in mgm. per gm. mouse.]

Diet.	Fatal dose of propionitrile.	
	Recovered.	Died.
a. Cakes	....	0.024
b. Potassium iodide, 0.02+4	0.030	

Potassium iodide caused a distinctly increased resistance to propionitrile, and the effect was greater than in the case of the bread-fed mice.

3. Experiments with Morphine.

(*a*) EXPERIMENTS ON MICE.

Diet has a distinct effect upon the resistance of mice to morphine as is shown by the following experiments:

Series 99. (June, 1908.) (x, 172.)

Mice were fed as below for about 75 days.[b]

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[a] Other members of these groups were used in experiments with acetonitrile. (series 87).

[b] These mice were the unused ones and, to a less extent, the survivors of those used in series 17 and 96.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of morphine sulphate.	
		Recovered.	Died.
a. Ham, lean	Increased 26 per cent	0.21	0.22
b. Milk, raw	Increased 13 per cent	.21	.22
c. Milk, boiled	Increased 25.6 per cent	.27	.28
d. Egg yolk	Increased 39 per cent	.33	.35
e. Liver (hog), boiled	Increased 10.5 per cent	.30	.40
f. Cheese	Increased 14.6 per cent	.35	.37
g. Oats	Increased 22.9 per cent	.35	.38
h. Bread	Increased 16.4 per cent	.40	.42
i. Bread and oat ash[a]	Increased 5.7 per cent	.37	.37
j. Potatoes	Decreased 7.9 per cent	.36	.40
k. Egg white, and bread, equal parts.	Increased 6.2 per cent	.50	.52
l. Egg white, and oatmeal, equal parts.	Increased 13 per cent	.55	....

[a] The mice received daily the ash from about 9 gms. oats intimately mixed with the bread.

A comparison of the results of this series with those of series 17 and 92, when the mice of these groups were tested with acetonitrile and propionitrile, shows that the degree of susceptibility to the three poisons is very different, i. e., the susceptibility to each poison is specific and not dependent upon "general resistance."

The same fact is illustrated also by the following series:

Series[a] 100. (Mar., 1910.) (xii, 38.)

The mice were fed as below for about 6 weeks.

Diet.	Change in weight (average).	Fatal dose of morphine sulphate.	
		Recovered.	Died.
a. Milk, boiled	Increased 4.65 per cent from 12.33 to 12.90 gms	0.28	0.35
b. Corn meal and water	Decreased 7.54 per cent from 12.33 to 11.40 gms	....	.40
c. Corn meal and boiled milk	Increased 27.33 per cent from 12.33 to 15.70 gms	.35	.45
d. Corn meal and oat extract	Decreased 3.56 per cent from 12.33 to 11.80 gms	.52	.55
e. Bread (C)	Increased 4.46 per cent from 12.33 to 12.88 gms	.65	.75
f. Corn meal and raw milk	Increased 37.14 per cent from 12.33 to 16.91 gms	.70	1.00
g. Bread (S)	None (12.24 gms)	.70	.90
h. Milk, raw	Increased 3.16 per cent from 12.33 to 12.72 gms	.75	.80

The effect of the different foods in altering, in a specific manner, the resistance of the mice to morphine is very evident from these experiments; this is especially marked when the results are compared with those of series 26 in which the poison used was acetonitrile. Thus with acetonitrile the mice receiving the raw milk were the least resistant; with morphine they were the most resistant. Mice receiving one brand of bread (C) were at least four times as resistant to acetonitrile as those receiving another brand (S); no such difference was observed in regard to morphine.

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[a] These mice were the unused ones and the survivors of those used in series 26.

The following experiments, although not numerous and perhaps in some cases not sufficiently controlled, may be quoted; the essentially negative results obtained in some cases are of some interest:

Series 101. (Mar., 1908.) (x, 112.)

The mice were fed on the following diets for about 50 days:

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of morphine sulphate.	
		Recovered.	Died.
a. Beans, "navy" (boiled)	Decreased 3 per cent	0.20	0.23
b. Peanuts (raw)	Decreased 7 per cent	.25	.30
c. Beef (boiled)	Increased 7 per cent	.28	.30
d. Corn	Decreased 11.4 per cent		.36

Series 102. (Mar., 1907.) (vi, 126.)

The mice were fed as below for 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of morphine sulphate.	
		Recovered.	Died.
a. Cakes	Decreased 7.7 per cent	0.38	0.43
b. Prostate 0.2+4	Decreased 14.2 per cent		.30

Series 103. (July, 1907.) (vii, 30.)

The mice were fed as below for 10 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of morphine sulphate.	
		Recovered.	Died.
a. Cakes	Increased 4.7 per cent	0.43	0.45
b. Parathyroid 0.05+4	Decreased 6.1 per cent	.20	.30
c. Prostate, 0.1+4	Increased 2.6 per cent	.31	.34

Series 104. (Nov., 1905.) (vi, 95.)

The mice were fed as below for 8 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of morphine sulphate.	
		Recovered.	Died.
a. Cakes	Decreased 0.9 per cent	0.24	0.26
b. Pituitary, 0.1+4	Decreased 1.3 per cent	.15	.20
c. Parathyroids, 0.2+4	Decreased 1 per cent		.35

Series 105. (Oct., 1908.) (xi, 42.)

The mice were fed as below for 9 days.

[Dose in mgm. per gm. mouse.]

Diet.	Change in weight (average).	Fatal dose of morphine sulphate.	
		Recovered.	Died.
a. Cakes	Increased 0.8 per cent	0.21	0.22
b. Sulphur [illegible]	Decreased 0.8 per cent	.20	.21

SUMMARY. These series of experiments, although very incomplete, admit of certain general conclusions. Thus sulphur, which protects mice (and other animals) so markedly against acetonitrile, has no influence upon the toxicity of morphine. Prostate distinctly lowers the resistance of mice to morphine; thyroid[a] has the same action. Both prostate and thyroid increase the resistance of mice to acetonitrile, whereas they both lower that of rats to this poison. They also lower the resistance of rats to morphine. From these results it is evident that the physiological action of these two glands when given per os are in a number of respects similar. Parathyroid and pituitary lowered the resistance of mice to morphine; they had a similar effect upon the resistance of these animals to acetonitrile. Thus toward one poison their action is similar to that of thyroid; toward the other it is the reverse.[b]

The results of the experiments with milk are at first sight very contradictory; in series 99 both raw and boiled milk caused a low degree of resistance to morphine, that with the raw milk being lower than that with the boiled milk. In series 100 boiled milk caused a very low degree of resistance, whereas raw milk caused a very high resistance. On comparing these results with those obtained with acetonitrile with mice of the same groups (series 17 and 26) certain relations seem to appear. Thus in series 17 and 99 boiled milk caused a lower degree of resistance to acetonitrile and a higher resistance to morphine than did raw milk; in series 26 and 100 boiled milk caused a higher resistance to acetonitrile and a lower resistance to morphine than did the raw milk. In other words, milk affected the resistance of mice to acetonitrile and morphine in opposite ways.

Since thyroid also affects the resistance of mice to morphine and acetonitrile in opposite ways, these results suggest that some of the effects of milk upon the resistance of mice to morphine may be due in part to certain effects of the milk upon the thyroid; there is no reason, however, to suppose that such an effect has more than a very subordinate part in determining the resistance to morphine.

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[a] Hunt and Seidell, Bull. 47, 1909, Hygienic Laboratory, Studies on Thyroid, I.

[b] These conclusions are expressed with considerable reserve, for the experiments were not numerous.

(b) EXPERIMENTS ON RATS.

That diet has some influence upon the toxicity of morphine for rats is indicated by the following experiments:

Series 106. (Feb., 1908.)

Thirty-four rats of approximately the same age and weight were divided into two groups of 17 each. One group was placed upon an exclusive diet of bread and water and the other group upon a diet of oats and water.

The following changes in weight occurred:

Date.	Average weight.	
	Bread.	Oats.
1907.	*Gms.*	*Gms.*
December 12	61.0	62.0
December 23	63.0	63.0
1908.		
January 3	66.0	62.0
January 17	74.0	62.0
January 23	78.0	73.0
February 10	84.6	81.3
February 17	102.2	89.8

The resistance of the rats of each group to morphine sulphate was now determined; the results were as follows:

Bread and water:
- One rat recovered from 0.3 mgm. morphine sulphate per gm. animal.
- One rat recovered from 0.31 mgm. morphine sulphate per gm. animal.
- One rat died from 0.33 mgm. morphine sulphate per gm. animal.
- One rat died from 0.35 mgm. morphine sulphate per gm. animal.
- One rat died from 0.40 mgm. morphine sulphate per gm. animal.

Oats and water:
- One rat recovered from 0.3 mgm. morphine sulphate per gm. animal.
- One rat recovered from 0.37 mgm. morphine sulphate per gm. animal.
- One rat recovered from 0.40 mgm. morphine sulphate per gm. animal.
- One rat died from 0.41 mgm. morphine sulphate per gm. animal.
- One rat died from 0.45 mgm. morphine sulphate per gm. animal.

Thus the resistance of the bread-fed rats was distinctly less than that of the oats-fed ones.

Potassium iodide to the extent of 0.1 per cent was added to the water and the above diets continued for 2 weeks, when the resistance of the rats to morphine was again tested; the results were as follows:

Bread and 0.1 per cent solution of potassium iodide:
- One rat recovered from 0.2 mgm. morphine sulphate per gm. animal.
- One rat recovered from 0.22 mgm. morphine sulphate per gm. animal.
- One rat died from 0.23 mgm. morphine sulphate per gm. animal.
- One rat recovered from 0.24 mgm. morphine sulphate per gm. animal.
- One rat died from 0.26 mgm. morphine sulphate per gm. animal.
- One rat died from 0.28 mgm. morphine sulphate per gm. animal.

Oats and 0.1 per cent solution of potassium iodide:

One rat recovered from 0.2 mgm. morphine sulphate per gm. animal.
One rat recovered from 0.22 mgm. morphine sulphate per gm. animal.
One rat died from 0.26 mgm. morphine sulphate per gm. animal.
One rat died from 0.31 mgm. morphine sulphate per gm. animal.

The resistance of the rats of both groups to morphine sulphate was thus distinctly diminished by the administration of potassium iodide. The effect of the latter is thus similar to that of thyroid[a] and the explanation is probably the same, in part at least, as that of the diminished resistance to acetonitrile of guinea pigs to which iodides have been administered, viz, that it is an effect exerted through the thyroid gland.[b] It is of interest to note that, although the fatal dose of morphine for both groups of rats, after the administration of potassium iodide, was the same, the increase in the susceptibility of the group which had received the diet of oats and water was relatively greater than that of the other group.

Series 107. (Mar., 1910.) (xii, 22.)

Rats were fed as below for about 7 weeks.

[Dose in mgm. per gm. rat.]

Diet.	Change in weight (average).	Fatal dose of morphine.	
		Recovered.	Died.
a. Bread, 2 parts; olive oil, 1 part.	Decreased 4.5 per cent (from 63.00 to 60.14 gms.)		0.27
b. Egg, whole	Increased 95.7 per cent (from 63.00 to 123.3 gms.)	0.27	.30
c. Oats	Increased 23.1 per cent (from 63.00 to 77.57 gms.)	.27	.31
d. Bread (C)	Increased 36.7 per cent (from 60.14 to 82.28 gms.)	.32	.35
e. Corn meal	Increased 16.7 per cent (from 63.00 to 73.57 gms.)		.42

Here again there were distinct differences in susceptibility to morphine; these did not seem to have any relation to changes in rate of growth.

SUMMARY.

1. A restricted diet markedly increases the resistance of certain animals to acetonitrile.

2. Guinea pigs upon a limited diet excrete a smaller percentage of the cyanogen of acetonitrile as sulphocyanate than do those upon an unrestricted diet. This result is interpreted as showing that certain specific processes of metabolism are retarded in partial inanition.

3. Diet has a marked effect upon the resistance of animals to certain poisons; the resistance of some animals may be increased forty-fold by changes in diet.

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[a] Hunt and Seidell, Bull. 47, Hygienic Laboratory, p. 77.

[b] Hunt, Journ. Am. Med. Ass., 1907, 49, p. 1323.

4. Certain diets, notably dextrose, oatmeal, liver, and kidney, greatly increase the resistance of mice to acetonitrile; their effect is similar in this respect to the administration of thyroid.

5. The effect of an oatmeal diet in increasing the resistance of certain animals to acetonitrile is probably due in part to a specific effect of the diet upon the thyroid gland; this is an illustration of how an internal secretion may be modified in a definite manner by diet.

6. Diet has, in certain cases, a marked effect upon the reaction of animals to iodine compounds; this effect is probably exerted largely through the thyroid. The condition of the latter is more important than the chemical form in which the iodine is administered.

7. Certain diets (notably eggs, milk, cheese, and various fats) greatly lower the resistance of certain animals to acetonitrile; their effect is the opposite of that of thyroid.

8. Several glands (notably prostate, ovaries, and testes) have an effect upon the resistance of animals to poisons similar to but much less marked than that of thyroid. Other glands (thymus, parathyroid, suprarenals) have either no effect or an effect opposite to that of thyroid.

9. The resistance of animals to propionitrile is markedly influenced by diet.

10. Diet causes distinct but not very marked differences in the resistance to morphine.

11. Season has an important effect upon the resistance of animals to certain poisons; in some cases these effects seem to depend upon seasonable variations in the activity of the thyroid.

12. The experiments show that foods such as enter largely into the daily diet of man have most pronounced effects upon the resistance of animals to several poisons; they produce changes in metabolism which are not readily detectable by methods ordinarily used in metabolism studies. The ease and rapidity with which certain changes in function are caused by diet are in striking contrast with the essentially negative results obtained by the chemical analyses of animals fed upon different diets.[a]

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[a] Compare Steinitz, Jahrb. f. Kinderheilk., 1904, 59, p. 447; Mendel, Biochemische Zeitsch., 1908, 11, p. 281.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

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